A SYNERGISTIC EFFECT OF ASBESTOS AND SMOKING IN LUNG CANCER: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Thesis entitled "A Synergistic Effect of Asbestos and Smoking in Lung Cancer: A Systematic Review and Meta-analysis"

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ABSTRACT

The main aim of this study was to reconcile and combined the result from observational studies investigating on asbestos exposure and cigarette smoking to develop lung cancer risk. Lung cancer is a leading cause of death worldwide. Cigarette smoking and asbestos exposure are known causes of the cancer. At present, there are many epidemiological studies investigating the effects of co-exposure of asbestos and smoking on lung cancer development. However, those studies showed conflicting results. Thus, we conducted a systematic review and meta-analysis, providing a quantitative estimate of the increased risk of lung cancer associated with asbestos exposure and cigarette smoking. I updated meta-analyses of published case-control and cohort studies exploring occupational and/or environmental asbestos exposure and tobacco smoking cause of lung cancer risk. Five electronic databases (PubMed, EMBASE, ISI web of knowledge, Scopus and TOXLINE) were searched from inception date to May, 2015 for observational study of lung cancer. We calculated pooled odds ratio (ORs), pooled relative risk (RRs) and 95% confidence intervals (CIs) using random effects model for the association of asbestos exposure and smoking with lung cancer. Lung cancer patients were compared with non-exposed asbestos and smoking controls, smoking controls and asbestos exposure controls. All cohort (N=7) and case-control (N=10) studies included in analyses were stratified by assessment of occupation/environmental exposure to asbestos and smoking. The summary estimates were observed for cohort studies; co-exposure of asbestos and smoking were associated with an increased risk of lung cancer compared with non-

exposed asbestos and smoking controls (RR 8.90; 95% CI 6.01-13.18), asbestos exposure groups (RR 2.72; 95% CI 1.67-4.40) and only smoking groups (RR 6.42; 95% CI 4.23-9.75). Case-control studies, co-exposure of asbestos and smoking were associated with an increased risk of lung cancer compared with non-exposed asbestos and smoking controls (OR 8.70; 95% CI 5.78-13.10), asbestos exposure groups (OR 1.70; 95% CI 1.31-2.21) and only smoking groups (OR 5.65; 95% CI 3.38-9.42). Synergy index (*S*) and multiplicative (*V*) were 1.44 (95% CI =1.26-1.77) and 0.91 (95% CI =0.63-1.30), respectively. Our results suggested that there was a statistical significance increased risk of lung cancer associated with co-exposure of asbestos and cigarette smoking in an additive synergism.

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ABBREVIATIONS

BaP	=	Benzo(a)pyrene
COPD	=	Chronic obstructive pulmonary disease
HMGB1	=	High-mobility group box-1
IARC	=	International Agency for Research on Cancer
ICD	=	International Classification of Diseases
NLRP3	=	Nod-like receptor-family protein 3
NNK	=	Nicotine-derived nitrosamine ketone
NOS	=	Newcastle-Ottawa scale
OR	=	Odd ratios
PAHs	=	Polycyclic aromatic hydrocarbons
ROS	=	Reactive oxygen species
RR	=	Relative risk
US-EPA	=	United States Environmental Protection Agency

CHARPTER I

INTRODUCTION

Statement of purpose

Lung cancer is the leading cause of death worldwide. Approximately, lung cancer death in both genders was 26-28 percent in USA in 2014 [1]. Cigarette smoking or tobacco smoke is the most important risk factor for the disease. Several compounds found in cigarette smoke [i.e. benzo(a)pyrene, PAHs (polycyclic aromatic hydrocarbons)] are classified as a human carcinogen by the International Agency for Research on Cancer (IARC) [2]. Cigarette smoking related diseases are associated with cardiovascular diseases, chronic obstructive pulmonary disease (COPD), emphysema, coronary heart disease and lung diseases [3]. Many countries such as Australia, Canada, China, Italy, Russia, and The USA has been classified as high tobacco consumption (more than 20 cigarettes per smoker per day) [4]. Moreover, there are other occupational exposures that are associated with lung cancer (i.e. arsenic, radon, diesel smoke gas and asbestos) [5, 6].

Asbestos is a group of silicate mineral fibers, primarily exerting its toxicities by inhalation [7]. Asbestos is classified as Group 1 human carcinogen by IARC [8]. Asbestos can be divided into two groups: serpentine and amphibole. Serpentine has only chrysotile. Amphibole has five distinct chemicals; those include crocidolite, actinolite, amosite, tremolite and anthophyllite. Scientific reports concerning adverse effects of asbestos were documented from the 1900 onwards. Several epidemiologic studies of asbestos exposure in mining and lung diseases were reported [9, 10, 11, 12]. Asbestos-related diseases are comprised of asbestosis, lung cancer and mesothelioma. Mesothelioma, a cancer of mesothelial cells, is a rare form of lung cancer originating in lining of the lung plural, pericardium and peritoneum. The disease has been used as a marker of asbestos exposure [13]. However, onset mesothelioma delayed more than 30-40 years post exposures [14]. As a consequence the prolonged onset of mesothelioma, it has been predicted that prevalence cases of mesothelioma will be continued and expected to reach its peak in the 21st century [15]. There is evidence

demonstrating that asbestos can lead to lung diseases both in laboratory animals [9, 16] and humans [10, 11, 17]. In humans, workers exposed to asbestos have a higher risk in developing lung cancer than the non-exposed population. A number of observational studies demonstrate that an interactive effect between asbestos exposure and cigarette smoking was additive [18], or more than additive [19] or multiplicative [20, 21]. However, the type of interaction between asbestos and smoking is still inconclusive.

Objectives of the study

1. To examine the interaction between exposure to asbestos and smoking on lung cancer risk.
2. To specify types of interaction whether or not the interaction between the two is either additive or multiplicative synergism.
3. To compare subgroup analysis effects of cigarette smoking and asbestos on lung cancer development.

Expected output of the study

1. The interactive effect of asbestos exposure and cigarette smoking will be identified.

Expected outcome

1. To verify the theory of interaction about co-exposure of asbestos and smoking.
2. The results of this study can be applied in risk assessment of asbestos and smoking and in a policy making process.

CHAPTER II

LITERATURE REVIEW

Asbestos

Asbestos is a group of naturally occurring mineral fibers. It has been used manufacturing product more than 3,000 products because of physical properties to resist heat and corrosives. Asbestos is commonly divided into two groups: (i) the serpentine group which exclusively composed of chrysotile; and (ii) the amphibole group comprising of five members including crocidolite, amosite, tremolite, actinolite and anthophyllite (Figure 1).

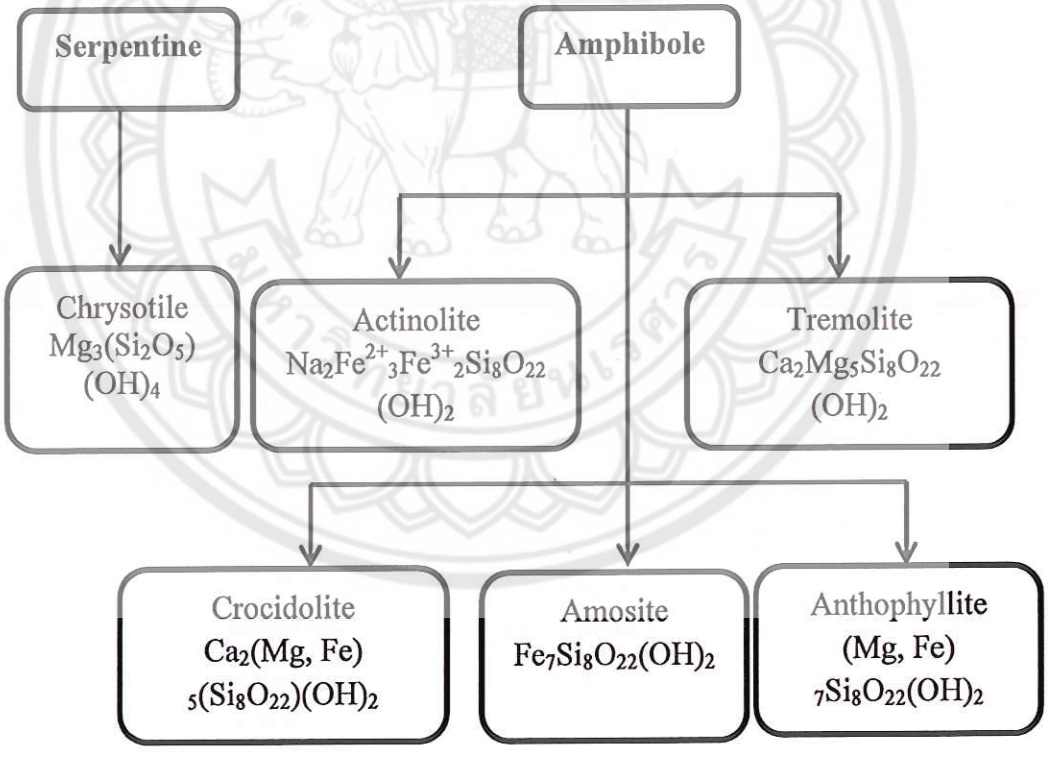
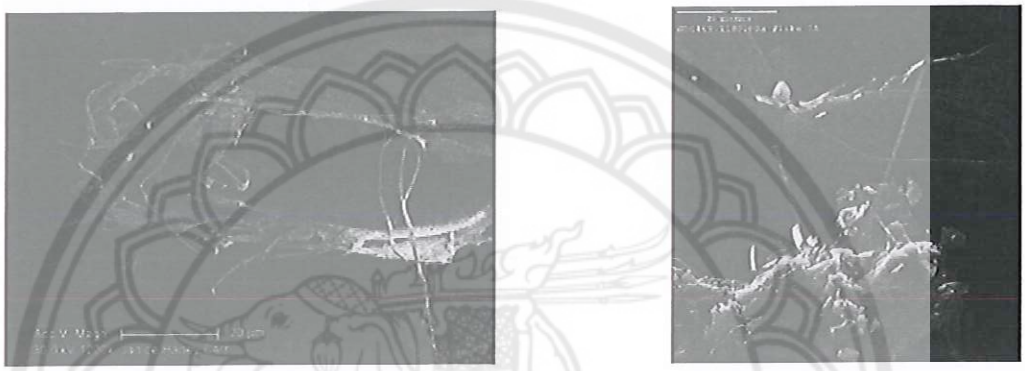


Figure 1 Flow diagram of asbestos types and chemical properties

Electron microscopic characteristic of chrysotile and amphibole asbestos is depicted in Figure 2A and 2B. For chrysotile, it contains magnesium (Mg) and silicon (Si) while amphibole asbestos has ferrous (Fe2+) and ferric ion (Fe3+) which can

induce an ion oxidation and lead to an oxidative stress via the Fenton reaction. A Fenton reaction is a process which generated free radicals. Fenton reaction has played an important role in biology of amphibole affecting on lung diseases.

Physical characteristic of serpentine is curly and more flexible than amphibole (Figure 2A). The amphiboles are likely straight-needle shape and can be more harmful to tissue than serpentine can (Figure 2B) [7, 8].



(A) (B)

Figure 2 (A) Chrysotile asbestos (B) Amphibole asbestos

Source: The global spread of asbestos [22]

Since asbestos has an excellent physical characteristics (i.e. highly resistant to heat), it has been widely in various kinds of heat resistant products [e.g. friction material (automotive brake shoes/pads and clutch plates), cement construction materials (roofing, cement pipes and shingles) and other insulation products]. However, its worldwide use has exponentially declined since 1980s, but asbestos is still in use in some countries for example China, India, Kazakhtan and Thailand [23].

Countries with high consumption asbestos mostly are located in Asia [23]. In 2013, China, Russia and India were the top three countries of the highest asbestos consumption rate (Figure 3). In South East Asia, Indonesia, Vietnam and Thailand import and produce many products/materials containing asbestos. This region use a large amount of asbestos, however, there was few reports of asbestos-related diseases in South East Asia.

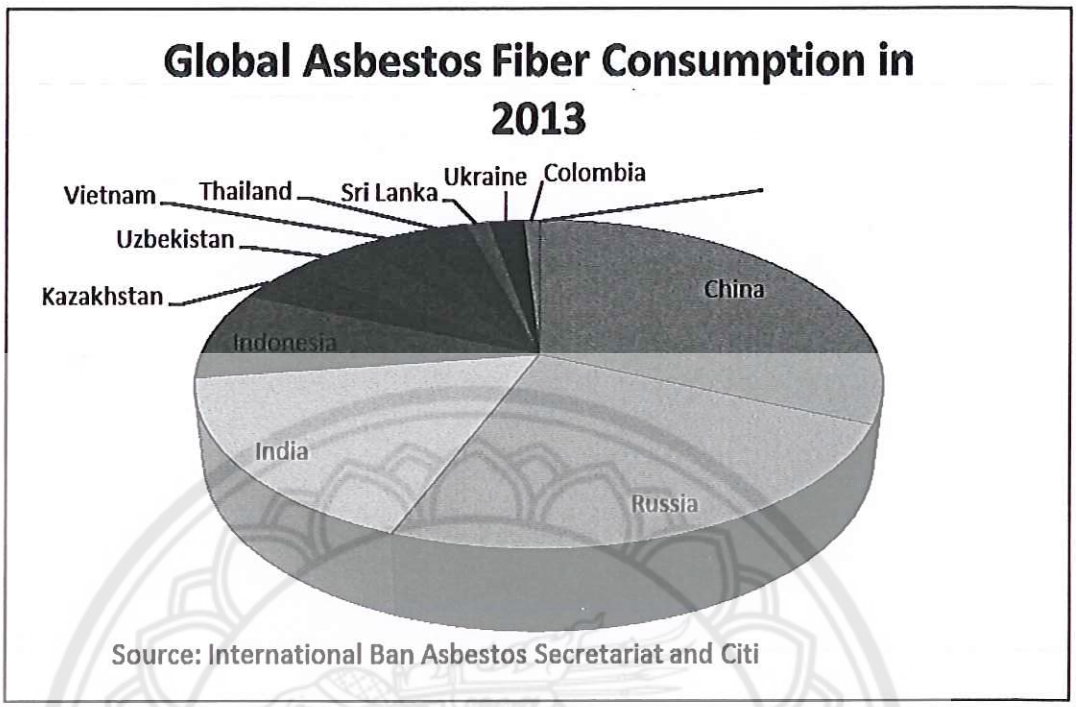


Figure 3 Diagram of global asbestos fiber consumption in 2013

As previously mentioned, Thailand is continually imported chrysotile asbestos but there are no asbestos mine. The imported asbestos was from Russia, Brazil and Canada. Asbestos has been imported for more than 30 years. Imported amount of asbestos to Thailand is about 100,000 tons between 1988 and 1997. During 2002-2005, the imported asbestos was at its peak (more than 150,000 tons). At present, trend of asbestos use in Thailand is decreasing. Since 2007, imported asbestos is less than 100,000 tons [23]. Nowadays, the serpentine is only type of asbestos permitted to use in the country. Chrysotile, which accounts for over 90% of the production of asbestos, is acclaimed nontoxic asbestos. Conversely, it has been reported that a long chrysotile can cause certain lung diseases (i.e. asbestosis, lung cancer) [24]. Therefore, public hazard from asbestos is still eminent in many countries. Most countries in Asia have been establishing a public policy for banning or controlling the use of asbestos, especially serpentine asbestos. A prediction of malignant mesothelioma or asbestos-related diseases was expected to reach its a peak at around year 2020 [25]. Determination of public policy for banning asbestos use

according to concerning asbestos-related health problems are still ongoing process in developing countries.

Asbestos and lung diseases

It has been well known since early 1940s that long-term exposure to asbestos can cause lung diseases [14]. Workers who had history exposed to asbestos had a higher risk to have mesothelioma [11, 12, 17, 21, 26]. As a result, IARC (International Agency for Research on Cancer) classified asbestos as a Group 1 known human carcinogen [7, 8]. The United States Environmental Protection Agency (US-EPA) also classified asbestos as a Class A known human carcinogen [27].

According to asbestos mining at Wittenoom, Western Australia during 1960-1990s, The first case of mesothelioma was reported in 1962 [13, 14]. Although, the latency period of developing asbestos-related diseases (malignant mesothelioma and lung cancer) is approximately 30-40 years from an initial asbestos exposure but most patients died within 9-12 months after their diagnosis [28]. Moreover, asbestos can also cause other diseases including gastrointestinal cancer, ovarian cancer and bladder cancer [29]. Recently, malignant mesothelioma cases were reported in Thailand [30]. This study is case reports of 2 cases of asbestos-related diseases (asbestosis and malignant mesothelioma).

The asbestos-related lung diseases can be divided into three types. Those include 1) asbestosis, 2) lung cancer, and 3) mesothelioma. Asbestosis, a non-cancerous of the lungs, is caused by inhaling asbestos fibers. The inhaled fibers trigger an inflammatory process, irritate lung tissues and cause fibrosis of the lining of the lung surface. The latency period of asbestosis is approximately 10–20 years. Lung cancer is a one of the diseases associated with cigarette smoking. There are two major types of lung cancer. Those are 1) the small cell lung cancer (SCLC), and 2) non-small cell lung cancers (NSCLC). The latency period for these cancers are 15–30 years. Lastly, mesothelioma is a rare form of cancer. The hallmark of mesothelioma includes exposure to asbestos fibers, erionite and the siman virus 40 (SV40).

Asbestos exposure from occupational and environmental settings

Adverse health effects from asbestos exposure are usually found in occupational settings. Occupations with a higher risk in asbestos exposure include miners, insulators, boilermakers, shipyard workers, textile workers, and builders.

Routes of asbestos exposure are inhalation and oral exposure via drinking water and eating contaminated foods [8]. Apart from the occupational exposure, environmental exposures of asbestos are also documented [26, 31, 32]. Plausible environmental exposures of asbestos are various. For example, 1) laundry which exposed to asbestos from asbestos contaminating clothes; 2) neighbors who nearby asbestos mines or industries; and 3) household exposure from asbestos-containing materials use. The Goswami, et.al., 2013 indicated that the risk of mesothelioma for persons domestically exposed is greater than five-fold compared to non-exposed populations [33]. In addition, some exposed populations either from occupational or from domestic asbestos exposure was not found asbestos-related diseases because they had differently eliminated asbestos from lung. A clearance of asbestos from the lung is an important factor for understanding of biodurability of asbestos.

Clearance of asbestos from the body

After inhaled into the lungs, the fibers can translocate to alveoli and lung cavity shortly after an initiation of exposure. If there are many fibers depositing into lungs, those fibers can lead to move and migration of leukocytes, thereby initiating inflammatory responses. Whether or not the included fibers are decomposed or cleared from the lung by macrophages depending on fiber types, fiber size and amount of asbestos fibers exposure.

For both serpentine and amphiboles, it takes a long time to be eradicated from human lungs. Due to the physical properties of amphiboles, these fibers have a double chain of tetrahedral silicate which makes it persistent in the human lungs. While the structure of chrysotile is a soluble magnesium layer, it is readily attacked a milieu at pH 4-4.5 inside the macrophages. Although, the studies [24, 34] reported that biopersistence of chrysotile is less prolonged compared to amphibole, they have similar carcinogenic effects on human lungs.

Consequently, small or large fibers were also considered. Small fibers (less than 5 μm in length) can be easily eliminated by alveoli macrophages. Long fibers (more than 20 μm in length) were deposited on lung bifurcations and hardly removed. They may be attributed to an increased ability to penetrate the lung plural cavity that is target sites of origin of malignant mesothelioma. However, asbestos fibers are longer than 200 μm, they cannot be discarded from macrophages because of fibers having

lengths greater than the macrophage diameter. A long-thin of amphibole can be penetrated the peripheral lung more readily than chrysotile whereas the curly fibers of chrysotile can be intercepted at airway bifurcations of lungs [35].

Moreover, an amount of asbestos exposure correlated with duration of exposure or period of doing jobs plays an important role the clearance of asbestos from the lungs. Studies reported that the populations who exposed asbestos were consumed on a large amount and prolonged time. They will have a higher risk of lung disease than those who were not [12].

Although some studies [24, 34] reported that chrysotile asbestos is being less potent than amphibole. Results from Bernstein, et al. there was no difference in the potency of the two types regarding the induction of lung tumors [24]. There were evidences demonstrating that humans can develop lung cancer from exposure to chrysotile asbestos, when the exposure is high and sustained for long periods [36, 37]. It also suggests that the hazard may be low if even high exposures were of short duration.

Mechanism of Asbestos Toxicity

The differences in biopersitence of asbestos types were described above. Accordingly, bioactivities of these two asbestos fibers were found inconsistently. Amphibole types claimed that cause more harmful than serpentine because of fiber characteristics, biopersistence and toxicity. For amphibole type, it has chemical properties that danger to lung tissue according with Fe ion induced fenton reaction [38]. As known that amphiboles comprised of ferrous (Fe^{2+} ion) and ferric (Fe^{3+}ion). The effects of Fe ions can induce Fenton reaction and lead to oxidative stress, causing cell damage. Fenton's reaction equation showed below:

$$Fe^{3+} + O_2^{\bullet -} \rightarrow Fe^{2+} + O_2$$

$$\underline{Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH}$$

$$O_2^{\bullet -} + H_2O_2 \xrightarrow[Fe^{3+}]{} OH^- + {}^{\bullet}OH + O_2$$

Chrysotile asbestos is a far less potent carcinogen than amphibole. However, it is classified as type 1 carcinogen. A large number of chrysotile and prolonged exposures are influenced on cell changes and translocation of neutrophils for fibers elimination more likely to produce lung cancer from amphibole.

After inhaled asbestos into the lungs, some fibers will be removed by macrophages whereas most of the fibers remain in the lungs. When leukocytes are attracted into lung areas, this can initiate chronic inflammation. Recruited macrophage cells performed phagocytosis of the asbestos fibers. However, some fibers were not eliminated by macrophages called "frustrated phagocytosis" and then the macrophages release inflammatory cytokines (i.e. tumor necrosis factor-α, interleukin-1β, transforming growth factor-β and platelet-derived growth factor) and reaction species [39]. Among these cytokines, TNF-α plays an important role in the inflammatory process. It can activate nuclear factor-κB which leads to mesothelial cell survival and inhibits asbestos-induced cytotoxicity [40].

Yang, et.al, study [41] reported that both chrysotile and amphibolies exposures had similarly carcinogenic effective on human mesothelial cell. Thereby, this increases the expression of HMGB1 (high-mobility group box-1), leading to undergo necrotic cell death and promoting an inflammatory response (Figure 4). Both amphibole and chrysotile fibers can induce apoptosis and transformation of lung tissues [39].

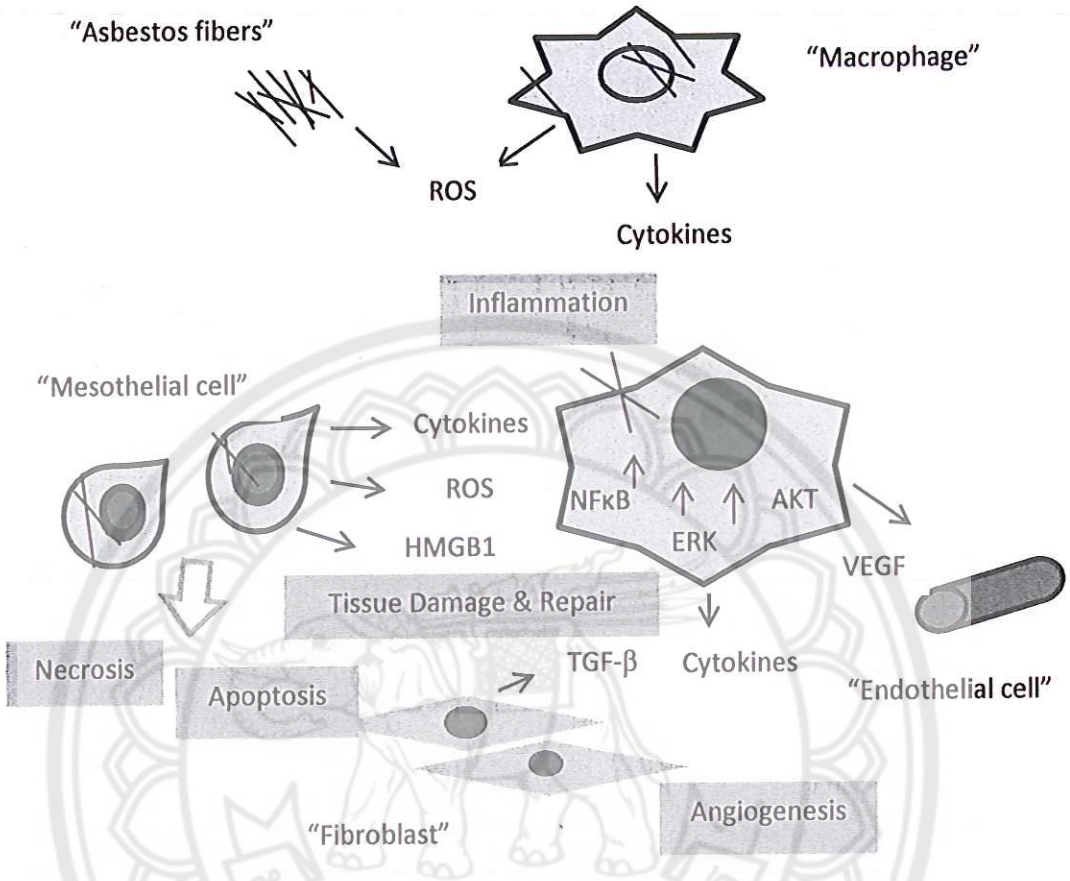


Figure 4 Mechanism aspects of asbestos-induced carcinogenesis

Source: Sekido [40]

In addition, asbestos fibers are a potent activator of inflammatory cascade. A Nod-like receptor-family protein 3 (NLRP3) inflammasome is a key mediator of chronic inflammation, potentially causing cancer [42]. The NLRP3 inflammasome can be changed, pro IL-1β, a pro-inflammatory cytokine to mature IL-1β (Figure 5). Activated inflammatory cytokines were released to eradicate asbestos fibers. They can produce cell damages and cell changes. Then, these cause cell transformation and necrosis. Theses inflammatory processes were placed repeatedly. Subsequently outcomes of these are lung diseases and cancer. This chronic inflammation leading to asbestos-induced lung cancer can be a long process, up to 30-40 years.

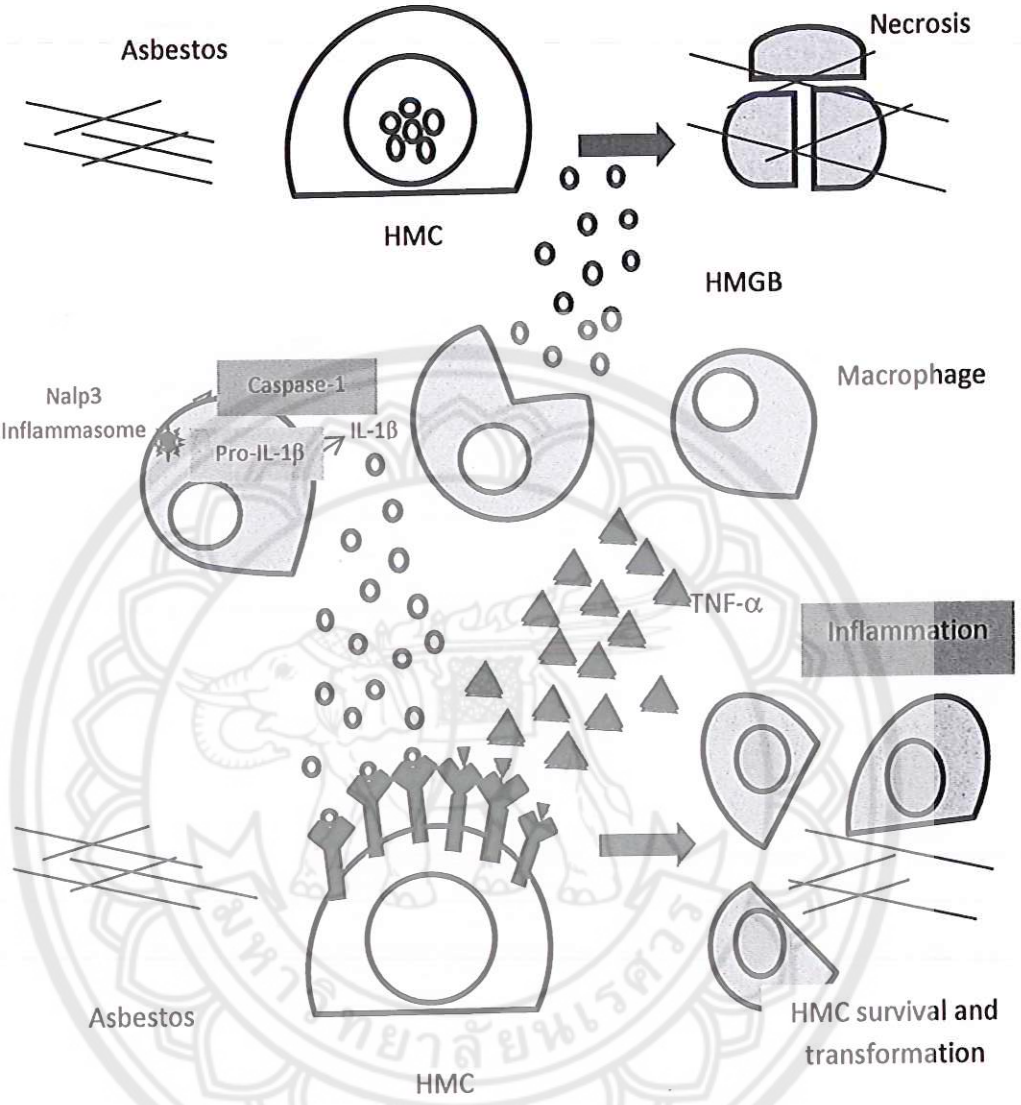


Figure 5 Role of Nod-like receptor-family protein 3 (NLRP3) inflammasome for carcinogenesis

Source: Carbone M. and Yang H. [41]

Fiber analysis techniques

There are several techniques that can be used to identify fibers in environmental and biological samples.

For environmental sample (i.e. an air sample from workplaces, manufactures, environment), midget impinge, impinge and membrane filter techniques can be used for collecting asbestos fibers that contaminated in the air. Then, detection of asbestos fibers from collection samples was used by microscopy.

For biological sample (i.e. lung tissues from biopsy and histology examination), the samples can be detected by light and electron microscopy. An electron microscopy comprises of scanning electron microscopy (SEM) and transmission electron microscopy (TEM). By the way, TEM is broadly distinguished on asbestos fibers.

With regard to the efficiency of light and electron microscopy, an electron microscopy can be detected the fiber 0.5 μm in length whereas light microscopy can be identified the fiber more than 5 μm in length. Eventually, no analytical methods could be detected the fiber less than 0.5 μm in length.

Additionally, exposure assessment of asbestos in epidemiological studies was typically performed by questionnaire/interview, job matrix exposure and expert assessment [43]. The only method is not perfectly for collecting data. Nonetheless, most epidemiological studies were commonly recommended in combining on two or more methods for setting on asbestos exposure assessment. Moreover, the potent of expertise can be identified and separated the fibers increasing of powerful and accurate on asbestos measurement method.

Cigarette smoking

There are over 5,000 compounds identified in tobacco smoke. Notably, polycyclic aromatic hydrocarbons (PAHs) [e.g.,benzo[a]pyrene (BaP)] and the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) are likely to play major roles in smoking related carcinogenesis. According to IARC, major compounds (BaP and NNK) in cigarette are classified as group 1 human carcinogen [2]. Both NNK and BaP can bind covalently to DNA and cause mutations. For NNK, NNN (N9-nitrosonornicotine) and aromatic amines can induce oxidative stress and cellular damages. From several epidemiological studies, smoking can increase the risk of developing lung cancer estimated 8-9 times compared to non-smokers [3]. In addition, the lung cancer risk in passive smokers was about 1-2 times compared to non-smoking subjects [42].

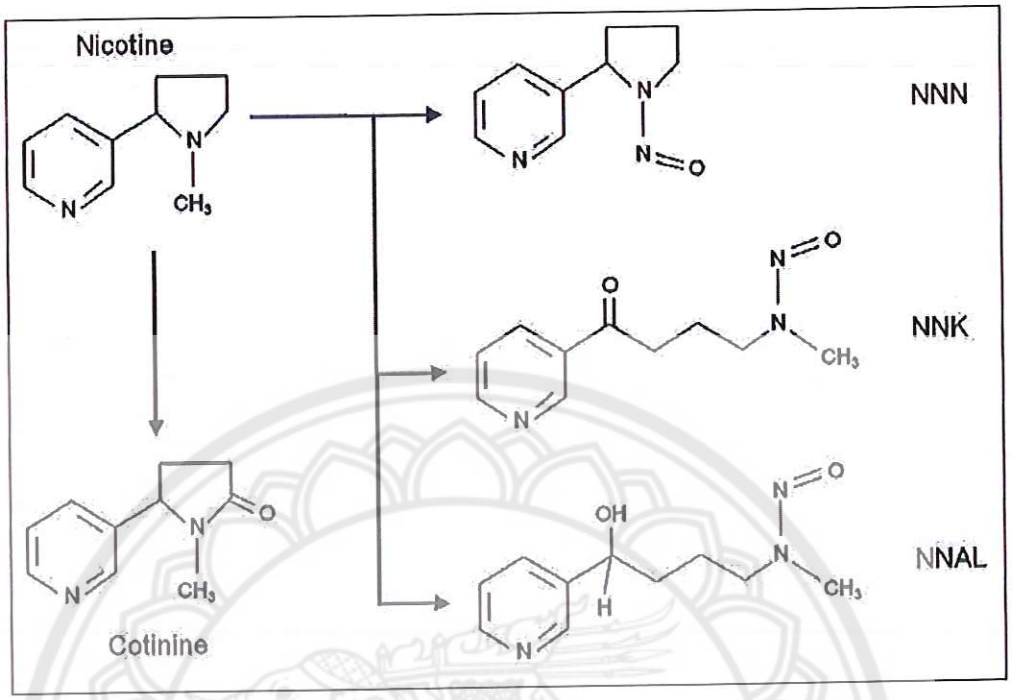


Figure 6 Chemical structures of nicotine and its derivative

Source: GW Warren and AK Singh [44]

Prevalence of cigarette smoke in worldwide and Thailand

Mechanism of cigarette smoking toxicity

As known that several compounds (e.g. BaP, NNK, and aromatic amines) in cigarette can adversely affect human health. Both NNK and BaP are metabolized by cytochrome P450 3A4. Their metabolites can bind covalently to DNA and cause DNA damages called "genotoxic effect" (Figure 7 showing metabolism of NNK and BaP, they bind to DNA).

For nicotine, several reports suggested that nicotine is responsible for addiction whereas it exert insignificant carcinogenic effects than its derivative. Nicotine cannot enhance tumor growth but nicotine and its nitrosated derivative NNK can cause DNA mutations directly [45].

A diagram on molecular mechanisms of genotoxic and non-genetoxic effects by cigarette smoke was summarized in Figure 7:

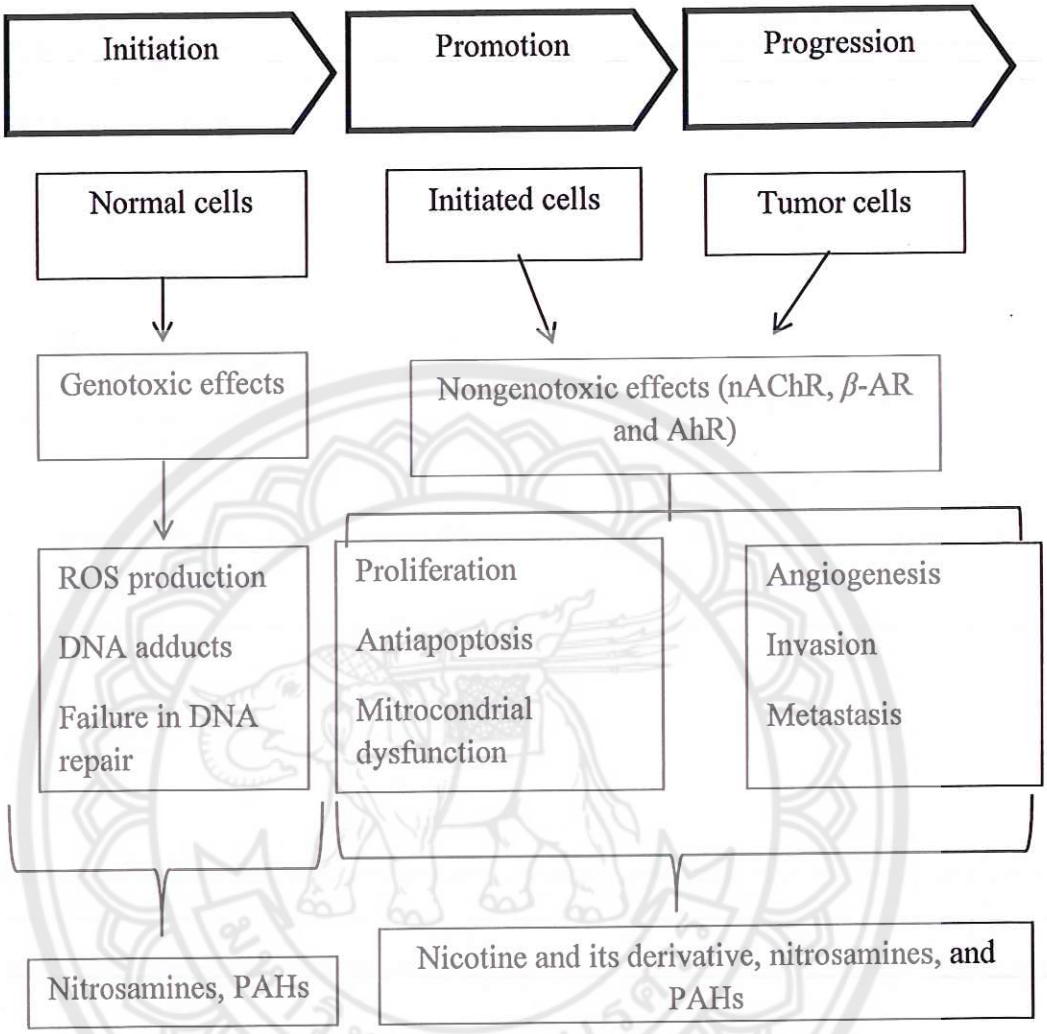


Figure 7 Flow diagram of cigarette compounds induced tumor growth

Source: Chen, et al. [46]

Lung cancer

Definition

Lung cancer is the uncontrolled growth of abnormal cells in lungs. These cells can multiply rapidly and turn into tumors. They can interfere with the function of the lungs and, ultimately, spread to other parts of the body.

Pathogenesis

There are two major histological types of lung cancer in the present classification, namely, small cell lung cancer and non-small cell lung cancer. Non-small cell lung cancer is divided into three subtypes i) squamous-cell carcinoma, ii)

large-cell carcinoma, and iii) adenocarcinoma. Aside from lung cancer, mesothelioma is a type of lung cancer that can develop in the membrane lining of the lungs and abdomen. The disease is used as a maker for asbestos exposure. Cigarette smoking, on the other hand, does not develop mesothelioma.

Prevalence of lung cancer

Worldwide people are smokers in both genders. An incidence in worldwide over 1.2 million are dead from lung cancer. Of these, estimate accounts for 80% of lung cancer in males and at least 50% in females [46]. Among males, death from lung cancer is ranked second following prostate cancer whereas lung cancer in females is a second death rate of subsequent breast cancer. In year 2014, it was forecasted that there would be 500,000 cases of lung cancer in the U.S. [45]. Nonetheless, the incidence rate in year 2015 was 43 cases per 100,000 persons. With this magnitude, several countries recommend a smoking cessation program in their countries.

Causes of lung cancer

There are several major causes of lung cancer. The most prominent causes included cigarette smoke, asbestos, radon, arsenic and coal dust [2, 47]. The epidemiological studies reported that lung cancer can be found in people who worked with asbestos and also smoking [18, 19, 20, 21]. Cigarette smoke is well-known to induce both types of cancer. A small cell lung cancer is mostly associated with frequent smokers.

Possible Mechanism of Co-Exposure to Asbestos and Cigarette Smoking

An individual of mechanism on asbestos related lung diseases can explain in several pathways. Asbestos fibers are directly got into lung. Small fibers (less than 5 μm) were further reached than large fibers. The long-thin fibers (more than 200 μm) have acclaimed that make incomplete phagocytosis of macrophages. The amphibole type has chemical properties (Fe ion) that were initiated Fenton reaction whereas a large amount of chrysotile was caused chronic lung inflammation. An asbestos exposure to continue developing lung cancer can be defined secretion of inflammatory cytokines which induced inflammatory process [48, 49].

For smoking, the compounds inside tobacco smoke had affected to lung. They can induce irritation and cause cancer. The mode of action of those chemicals in cigarette smoke described as following: polycyclic aromatic hydrocarbons (benzo(a)pyrene and Nicotine-derived nitrosamine ketone). Their metabolite compounds can activate multiple signaling pathways that contribute to lung cancer carcinogenesis such as cell proliferation and survival signals.

As a sequence, it is well-known that both exposure of asbestos and smoking were damaged on cells into lung. There are evidence reported that cigarette smoking can be interrupted clearance of asbestos [50]. The possible of mechanism on two exposures was explained on chronic inflammation association with cancer. Recent studies [51, 52, 53] suggested that both exposure of asbestos and smoking were powerfully originated reactive oxygen species (ROS). Then, they were triggered pro-inflammatory cytokines and activated releasing cell mediators. The effect caused cell proliferation and cell survival. Hence, inflammation is a hallmark of both asbestos exposure and cigarette smoking and is observed both in animals and humans. These are frequently associated with increased risk of cancers and slightly escalated on the two exposures by synergy. However, the mechanism of two exposures is still debated.

Asbestos and smoking interaction

In brief on biological interaction, when asbestos is inhaled into the lungs, its fibers can cause chronic inflammation for a long period of time. In addition, Fe ion in its fibers can induce prolonged Fenton's reaction, thereby, increase free radical production. Taken together, these can eventually induce lung cancer. On the other hand, carcinogens in cigarette smoke (i.e. BaP, NNK) can lead to mutations of DNA, thereby, cancer.

Since co-exposure of these two known human carcinogens is likely among populations, with their different modes of actions in carcinogenesis (chronic inflammation oxidative stress for asbestos and DNA damage for smoking), interactions between asbestos and smoking in lung cancer development are possible.
By statically interaction, while a person who exposed to two or more compounds/ chemicals at same time, results in health effects demonstrate that are greater than the sum of the effects of the individual chemicals called "synergism".

The nature of the joint effect of smoking and asbestos exposure on lung cancer mortality was investigated using two indices for interaction effects: the Synergy (*S*) on additive scale and Multiplicativity (*V*) indices on a multiplicative scale [54, 55]. A value of *S* greater than one indicates some degree of interaction between smoking and asbestos exposure on lung cancer risk, with a value of *S* equal to one indicating no interaction (that is, the effect of the two factors on risk is additive). For the second index, a value of *V* equal to one indicates a multiplicative interaction, whereas a value less than one indicate a less than multiplicative interaction. However, there is not a systematic review and meta-analysis using these statistics to test both hypotheses.

Therefore, this study aims for identify interaction between exposure to asbestos and smoking using systematic review and meta-analysis.

Measures of interaction on an additive scale and multiplicative scale

According to the observational studies, they were two dichotomous patterns for measurement. The two exposures divided into A and B:

1. OR_{A+B+} and/or RR_{A+B+} is the odds ratio/relative risk of disease if both A and B are present,
2. OR_{A+B-} and/or RR_{A+B-} is the odds ratio/relative risk of disease if A is present but B is absent, and
3. OR_{A-B+} and/or RR_{A-B+} is the relative risk of disease if A is absent but B is present.

Interaction on additive

For calculation on an additive scale [56], it has 3 equations as follow:

1. Relative excess risk due to interaction (RERI):

$$RERI = OR, RR_{A+B+} - OR, RR_{A+B-} - OR, RR_{A-B+} + 1$$

RERI = 0 refers to no interaction or exactly additive interaction (additivity);

RERI > 0 refers to positive interaction or more than additivity;

RERI < 0 refers to negative interaction or less than additivity.

2. Proportion attributable to interaction (AP):

$$AP = \frac{RERI}{OR, RR_{A+B+}}$$

AP = 0 refers to no interaction or exactly additivity;

AP > 0 refers to positive interaction or more than additivity;

AP < 0 refers to negative interaction or less than additivity.

3. Synergy index (S):

$$S = \frac{RR_{A+B+}, OR_{A+B+} - 1}{(RR_{A+B-}, OR_{A+B-} - 1) + (RR_{A-B+}, OR_{A-B+} - 1)}$$

S = 1 refers to no interaction or exactly additivity;

S > 1 refers to positive interaction or more than additivity;

S < 1 refers to negative interaction or less than additivity.

Interaction on multiplicative [54]

For calculation on a multiplicative scale, it was used an equation as follow:

$$V = \frac{OR, RR_0 * OR, RR_{A+S+}}{OR, RR_{A+S-} * OR, RR_{A-S+}}$$

$V = 1$ refers to no interaction on the multiplicative scale;

$V > 1$ refers to positive multiplicative interaction;

$V < 1$ refers to negative multiplicative interaction.

CHAPTER III

RESEARCH METHODOLOGY

The methodology of systematic review and meta-analysis has five processes to select the studied that met criteria as follow: 1) Define question, 2) Searching, 3) Study selection, 4) Data extraction and quality assessment, and; 5) Data analysis.

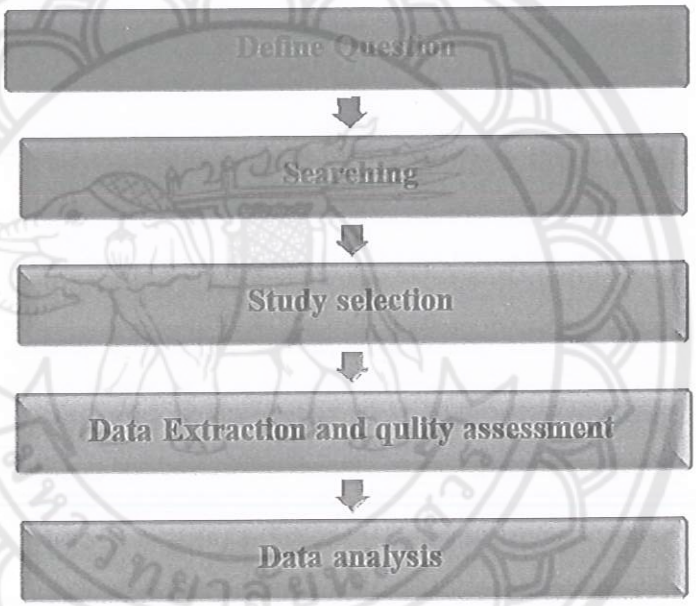


Figure 8 The processing of systematic review and meta-analysis

Search Strategy and study selection

A Comprehensive and systematic search was performed on the following databases: PubMed, Embase, Scopus, ISI Web of Knowledge, and TOXLINE databases from their inception until May 2015. Combinations of the following key words were used: asbestos, crocidolite, amosite, chrysotile, tremolite, actinolite, anthophyllite, cigarette, cigarette smoke, cigarette smoking, pipe, cigar, tobacco, tobacco smoking, lung cancer, mesothelioma, lung carcinoma, and lung adenocarcinoma. There was no language restriction. Only observational studies were

included. Additional studies were also hand-searched from bibliographies of the selected studies.

Table 1 Search terms used for identify relevant studies

Words	Search terms
Asbestos	"asbestos" OR "crocidolite" OR "amosite" OR "chrysotile" OR "tremolite" OR "actinolite" OR "anthophyllite"
Smoking	"cigarette" OR "cigarette smoke" OR "cigarette smoking" OR "pipe" OR "cigar" OR "tobacco" OR "tobacco smoking"
Lung cancer	"lung cancer" OR "mesothelioma" OR "lung carcinoma" OR "lung adenocarcinoma"

Inclusion and exclusion criteria

Studies were included if they met all of the following criteria: 1) original articles published in peer-reviewed journals; 2) human studies; 3) observational studies; 4) studies investigating associations between asbestos exposure and smoking with lung cancer, and; 5) studies reporting sufficient data for calculating odds ratio and relative risks. The studies that not meeting these inclusion criteria described above were excluded. If there were duplicate populations, only the studies providing the most details, more number of participants, followed populations for longer follow-up periods, or the most recently published were selected for meta-analysis. Two reviewers independently appraised titles and abstracts retrieved from the comprehensive searches. The controversial reviews were discussed and resolved by a third reviewer. If further details were required, the reviewers contacted the authors for more information.

Data Abstraction and Quality Assessment

Information extracted from each study included first author, publication year, geographic area, study type (hospital-based case-control, population-based case-control, nested case-control, retrospective cohort, prospective cohort, and cross-sectional), total number of cases, and controls, fiber type (chrysotile, crocidolite,

tremolite), industry type, measurement of asbestos and/or smoking exposure, asbestos exposure assessment method, definition of asbestos exposure and/or smoking, period of employment/exposure, measurement method (asbestos exposure, smoking), and classification of outcome. The Newcastle-Ottawa quality assessment scale (NOS) was used to assess the quality of the selected observational studies. The categories of NOS was based on selection of participants, comparability of study groups, and the exposure of interest (case-control studies) or outcome of interest (cohort studies) [57].

Statistical Analysis

Subjects were characterized into four groups: non-exposure to asbestos and non-smoking (A-S-), asbestos-exposed and non-smoking (A+S-), non-exposed asbestos and smoking (A-S+), and asbestos-exposed and smoking (A+S+). The primary outcome of the pooled analysis focused on comparing the summary effect of lung cancer risk in people without asbestos exposure and non-smoking versus those without asbestos exposure, non-smoking, and co-exposure to asbestos and smoking as follows: (i) A+S- compared with A-S- (ii) A-S+ compared with A-S-, and (iii) A+S+ compared with A-S- and interaction between asbestos and smoking were evaluated using the Rothman Synergy Index [55]. Summary effect estimates were assessed discretely by averaging the natural logarithmic OR and/or RR weighted by their inverse variances. The pooled effect estimates were calculated using a random effects model by the method of DerSimonian and Laird [58]. Heterogeneity among selected studies was determined using the Q-statistic and *I*-squared tests [59]. *I*-squared (I^2) values of 25%, 50%, and 75% represented low, moderate, and high degrees of heterogeneity, respectively [60]. The meta-analysis of case-control and cohort studies were conducted separately due to differences in the nature of study design [61].

Subgroup analyses were performed according to the geographic area (Europe, America, others), asbestos type, study design (hospital or population, retrospective, prospective), and stratification of smoking level were used to assess the impacts of study characteristics on outcomes. Publication bias was quantified using funnel plot, Begg's test and Egger's test, where $p>0.05$ for both tests was considered to have no significant publication bias [62, 63]. All analyses were performed using STATA software V.10.1 (Stata Corp, College Station, TX, USA).

Determination of interactive effect

The joint effect of exposure to asbestos and smoking was first examined by estimating odds ratio (ORs) and relative risk (RRs). To determine whether co-exposure to asbestos and smoking is an additive and multiplicative scale, the synergy (*S*) and multiplicative (*V*) indices were calculated as follow [55, 64]:

Synergy index (*S*)

$$S = \frac{X_{AS} - X_0}{X_A + X_S - 2X_0}$$

Multiplicative index (*V*)

$$V = \frac{X_0 \times X_{AS}}{X_A \times X_S}$$

Where X_0 is the odds ratio and/or relative risk for lung cancer among non-exposed to asbestos and non-smokers; X_A is the corresponding value for lung cancer among asbestos exposure in non-smokers; X_S is for lung cancer and smoking in those without asbestos-exposure; and X_{AS} is for lung cancer and co-exposure to asbestos and smoking. The synergy index (*S*) is an interaction on an additive scale. The interpretation is $S = 1$ suggests no interaction between asbestos exposure and smoking on lung cancer; $S > 1$ suggests a positive interaction (synergism); and $S < 1$ suggests a negative interaction (i.e., antagonism). For the multiplicative index (*V*), it can be interpreted as either: when $V = 1$, there is no interaction on the multiplicative scale; when $V > 1$, the multiplicative interaction is positive; or when $V < 1$, it is negative. Confidence intervals (CIs) were calculated using the method of Rothman, and Andersson, et al. [55, 64, 65].

CHAPTER IV

RESUTS AND DISCUSSION

Study 1: An interaction of asbestos and smoking

Study Selection

I identified 2,499 records of which 2,479 were duplicated, irrelevant, review articles, case reports, non-human or experimental studies, or lacked lung cancer outcomes or lacking control groups, and were excluded. Five additional publications meeting the inclusion criteria were added from the bibliographies of the retrieved articles (Figure 9). In the final review of 25 studies, we excluded 5 studies [37, 66, 67, 68, 69] due to duplicate populations, and 3 studies [17, 70, 71] had insufficient data. Only one by Kjuus, et al. [72] was selected of three articles [67, 68, 72] which analyzed the same data. Case-control studies by Bovenzi (1992 and 1993) [66, 73], the cohort studies of McDonald and Liddell [37, 74]; and cohort studies of Klerk and Reid [20, 69] also described the same populations of which the most recent [20, 73, 74] was selected. The Blot, et al. study [17] did not report smoking status in asbestos-exposed populations. Finally, the studies of Hilt et al. 1986, and Markowitz, et al. [70, 71] were excluded because numbers of controls were missing. Therefore, a total of 17 studies (10 case-control and 7 cohort studies) were included for meta-analysis. The 13 included studies were identified using the search terms, and another 4 studies derived from their bibliographies.

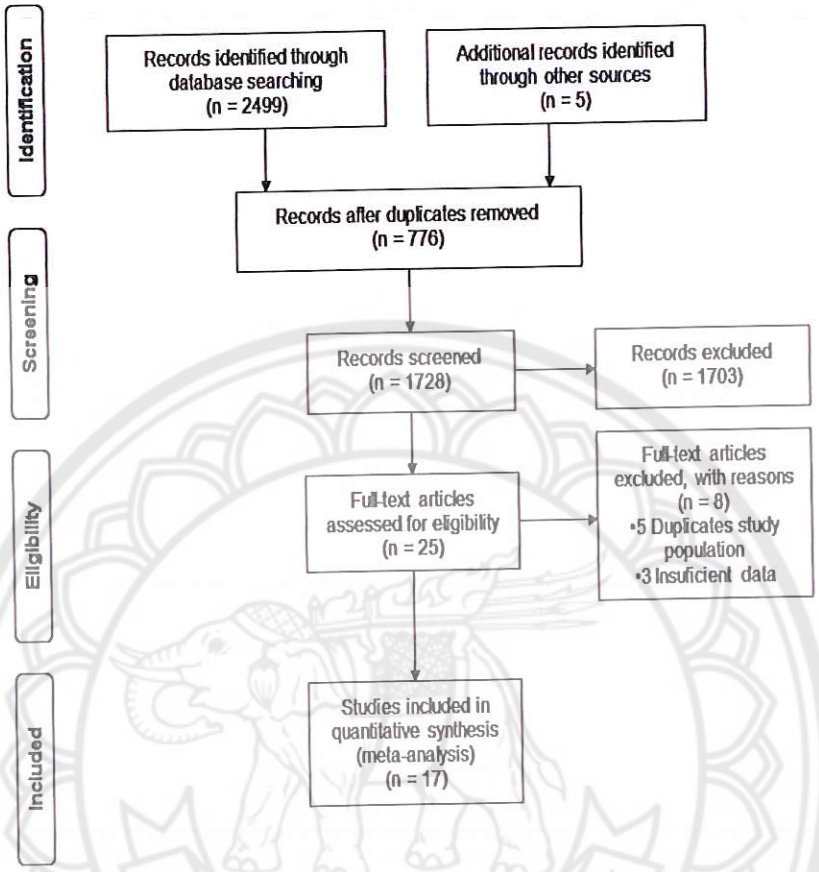


Figure 9 A Flow diagram of the systematic searching PRISMA

Study Characteristics

The characteristics and information of the included studies are shown in Table 2 and Table 3. The 10 case-control studies [11, 19, 21, 72, 73, 75, 76, 77, 78, 79], contained 10,223 participants in all of which 4,768 were population-based controls, and 1,128 hospital-based controls. Seven cohort studies [10, 18, 20, 36, 74, 80, 81] had an aggregate of 64,924 participants, comprising of the 3,316 cases and 61,608 controls. In all the included studies asbestos exposure was occupational. Where reported, the average participant age was approximately 60 (range 40-80 y) for case control studies. Some [75, 76] reported the type of asbestos used (tremolite or mixed asbestos), while the remaining eight [11, 19, 21, 72, 73, 77, 78, 79] did not categorize the asbestos (Table 2 and 3). The settings for the exposure was occupational, either asbestos mines (one study [75]), ship building/repair (two studies [11, 78]), textile production (one study [76]), and the remaining six [19, 21, 72, 73, 77, 79] studies

failed to specify. Environmental monitoring was measured by using the membrane filter method and were analyzed by phase contrast microscopy [19] but most studies relied on personal/telephone interview and/or questionnaire. Smoking habits of participants were quantified by personal/telephone interview and/or questionnaire. If the subject had already died, the appropriate information was sought from their next-of-kin or spouse (Table 4 and Table 5).

Quality Assessment

The methodological quality of case-control studies was summarized as a mean NOS of 6 (range 5-7) and a score of 6.7 (range 6-8) for cohort studies (Table 2 and Table 3).

Table 2 Characteristics of studies included in the meta-analysis (Case-control studies)

First author(year)	Location	Industrial type*	Asbestos type	Study design	Total population		NOS**
					Case (n)	Control (n)	
Case-control studies (n = 10)							
Martischnig(1977)[77]	United Kingdom	Not specified	Not reported	Hospital-Based	201	201	6
Blot(1978)[11]	Coastal Georgia, USA	Shipbuilding	Not reported	Hospital-Based	458	553	5
Blot(1980)[78]	Coastal Virginia, USA	Shipyard	Not reported	Population-Based	319	341	6
Pastorino(1984)[76]	Lombardy Northern, Italy	Manufacturing & textiles	Mixed	Population-Based	106	226	6
Kjuus(1986)[72]	Southern Norway	Not specified	Not reported	Hospital-Based	176	176	7
Dave(1988)[79]	Southeast Sweden	Not specified	Not reported	Hospital-Based	62	198	5
Bovenzi(1993)[73]	Northeast Italy	Not specified	Not reported	Population-Based	516	561	6
Luce(2000)[75]	New Caledonia, France	Mining & refining	Tremolite	Population-Based	103	110	6
Gustavsson(2002)[19]	Stockholm, Sweden	Not specified	Not reported	Population-Based	768	1519	6
Villeneuve(2012)[21]	8 locations, Canada	Not specified	Not reported	Population-Based	1618	2011	7

*All studies are occupational exposures, **NOS = Newcastle Ottawa-Scale

Table 3 Characteristics of studies included in the meta-analysis (Cohort studies)

First author(year)	Location	Industrial type*	Asbestos type	Study design	Total population		NOS**
					Case (n)	Control (n)	
Cohort studies (n = 7)							
Berry(1972)[81]	London, England	Asbestos factory	Not reported	Prospective	61	1678	6
Rubino(1979)[80]	Balangero mine, Italy	Mining	Chrysotile	Prospective	12	54	7
Liddell(1984)[74]	Quebec, Canada	Mining & milling	Chrysotile	Prospective	223	715	6
Berry(1985)[10]	London, England	Asbestos factory	Not reported	Prospective	66	1268	6
Reid (2006)[20]	Western Australia	Mining & milling	Crocidolite	Prospective	138	2595	7
Markowitz(2013)[18]	USA	Insulator	Not reported	Prospective	2760	55161	8
Wang (2013)[36]	China	Mining	Chrysotile	Prospective	56	137	7

*All studies are occupational exposures, **NOS = Newcastle Ottawa-Scale

Table 4 Descriptions of Asbestos Exposure of Included Studies (Case-control studies)

First author(year)	Measurement of exposure	Definition of asbestos exposure	
		Exposed	Non-exposed
Case-control studies (n = 10)			
Martischnig(1977)[77]	Questionnaire	Occupational history (work in asbestos manufacturing or used asbestos)	No occupational history
Blot(1978)[11]	Personal interview	Occupational history (work in shipbuilding or used asbestos)	No occupational history (never work in shipbuilding)
Blot(1980)[78]	Personal interview	Occupational history (shipyard)	No occupational history (never work in shipyard)
Pastorino(1984)[76]	Personal interview	Exposed to asbestos only	Exposed other carcinogenic chemicals
Kjuus(1986)[72]	Personal interview and questionnaire	Asbestos exposure at least 1 year or more and job title information	No exposure and no job title
Dave(1988)[79]	Self-administered questionnaire and telephone interview	Occupational history (works related to asbestos)	Occupational history (other works)

Table 4 (cont.)

First author(year)	Measurement of exposure	Definition of asbestos exposure	
		Exposed	Non-exposed
Case-control studies (n = 10)			
Bovenzi(1993)[73]	Personal interview	Occupational history (classified by job titles and asbestos exposure information)	No occupational history
Luce(2000)[75]	Personal interview	Occupational history (classified by expert assessment)	No occupational history
Gustavsson(2002)[19]	Questionnaire, telephone interview and environmental measurement	Occupational history and asbestos exposure > 0 fiber-years	No occupational history and asbestos exposure 0 fiber-years

Table 4 (cont.)

First author(year)	Measurement of exposure	Definition of asbestos exposure	
		Exposed	Non-exposed
Case-control studies (n = 10)			
Villeneuve(2012)[21]	Questionnaire	Occupational history (classified by concentration, frequency and reliability)	No occupational history

Table 5 Descriptions of Smoking of Included Studies (Case-control studies)

First author(year)	Measurement of exposure	Definition of smoking status	
		smoked	Non-smoked
Case-control studies (n = 10)			
Martischnig(1977)[77]	Questionnaire	14 cigarettes/day or more	0-14 cigarettes/day
Blot(1978)[11]	Personal interview	10 cigarettes/day or more	<1/2 pack/day and stopped smoking at least 10 years

Table 5 (cont.)

First author(year)	Measurement of exposure	Definition of smoking status	
		smoked	Non-smoked
Case-control studies (n = 10)			
Blot(1980)[78]	Personal interview	10 cigarettes/day or more	<1/2 pack/day and stopped smoking at least 10 years
Pastorino(1984)[76]	Personal interview	10 cigarettes/day or more	0-9 cigarettes/day
Kjuus(1986)[72]	Personal interview and questionnaire	10 cigarettes/day or more	0-9 cigarettes/day
Dave(1988)[79]	Self-administered questionnaire and telephone interview	>80 cigarette-years	0 cigarette-years
Bovenzi(1993)[73]	Personal interview	>1 cigarette/day	No smoked
Luce(2000)[75]	Personal interview	>20 pack-years	< 20 pack-years
Gustavsson(2002)[19]	Questionnaire and telephone interview	>1 cigarette/day	No smoked
Villeneuve(2012)[21]	Questionnaire	10 pack-years or more	< 10 pack-years

Table 6 Descriptions of Asbestos Exposure of Included Studies (Cohort studies)

First author(year)	Measurement of exposure	Definition of asbestos exposure	
		Exposed	Non-exposed
Cohort studies (n = 7)			
Berry(1972)[81]	Questionnaire	Occupational history	No occupational history
Rubino(1979)[80]	Environmental measurement	Occupational history (mining)	No occupational history
Liddell(1984)[74]	Environmental measurement	Cumulative exposure >100 fiber/year	Cumulative exposure 0-100 fiber/year
Berry(1985)[10]	Questionnaire	Occupational history	No occupational history
Reid (2006)[20]	Questionnaire and environmental measurement	Occupational history	No occupational history
Markowitz(2013)[18]	Clinical method (x-ray and spirometry)	Occupational history (insulation)	No occupational history
Wang (2013)[36]	Environmental measurement	Cumulative exposure >20 fiber-year/ml	Cumulative exposure <20 fiber-year/ml

Table 7 Descriptions of Smoking of Included Studies (Cohort studies)

First author(year)	Measurement of exposure	Definition of smoking status	
		smoked	Non-smoked
Cohort studies (n = 7)			
Berry(1972)[81]	Questionnaire	smoked	No smoked
Rubino(1979)[80]	Personal interview	smoked	No smoked
Liddell(1984)[74]	Questionnaire	>1 pack-years	0 pack-years
Berry(1985)[10]	Questionnaire/interview	smoked	No smoked
Reid (2006)[20]	Questionnaire	Smoked and ex-smoked < 20 years	No smoked and ex-smokers > 20 years
Markowitz(2013)[18]	Not reported	smoked	No smoked
Wang (2013)[36]	Questionnaire/interview	smoked	No smoked

Table 8 Descriptions of Outcome of Included Studies (Case-control studies)

Author(Year)	Case confirmation method	Diagnosis period	Lung cancer classification	Control matching	Period of exposure or employment
Case-control studies (n = 10)					
Martischnig(1977)[77]	By radiography, bronchoscopy or thoracotomy	1972-1973	Not reported	Age (±2 years)	1-5 years and 6 years and over
Blot(1978)[11]	By physician	1970-1976	ICD 8 162.1	Sex, race, age (±2 years)	6 months or more
Blot(1980)[78]	By physician	1976	ICD 162.1	Race, age, death year, city of residence	6 months or more
Pastorino(1984)[76]	By physician	1976-1979	Not reported	Age (±2 years)	6 months or more
Kjuus(1986)[72]	By examination of histology		ICD 162-163	Age (±5 years)	1979-1983
Dave(1988)[79]	Not reported	1980-1982	ICD 162-163	Age, sex	Not reported
Bovenzi(1993)[73]	By examination of histology, autopsy reports		ICD 9th 162	Age (±2 years)	Not reported

Table 8 (cont.)

Author(Year)	Case confirmation method	Diagnosis period	Lung cancer classification	Control matching	Period of exposure or employment
Case-control studies (n = 10)					
Luce(2000)[75]	By clinical, radiological and endoscopic	1993-1995	ICD for oncology topography code 160-162,148	Sex, age (±5 year)	Not reported
Gustavsson(2002)[19]	Not reported	1985-1990	ICD 7th 162.1	Age (±5 year) and year of inclusion study (1985-1990)	1969-1973
Villeneuve(2012)[21]	By examination of histology	1994-1997	ICD 9th 162	Age, sex	At least 12 months

Table 9 Descriptions of Outcome of Included Studies (Cohort studies)

Author(Year)	Case confirmation method	Diagnosis period	Lung cancer classification	Control matching	Period of exposure or employment
Cohort studies (n = 7)					
Berry(1972)[81]	By examination of histology	Not reported	ICD 162,163	Not reported	Men 1933-1955 Women 1936-1942
Rubino(1979)[80]	By physician	1957	ICD 7 162/163	Age (±1 year)	1930-1965
Liddell(1984)[74]	Not reported	Not reported	ICD 7th	Not reported	1966-1975
Berry(1985)[10]	By examination of histology	Not reported	The Office of Population Censuses and Surveys	Not reported	Men 1933-1955 Women 1936-1942
Reid (2006)[20]	By physician	2000 and 2002	ICD-0 2nd edition categories c33.9-c34.9	Sex, age (±5 years)	1979-2002

Table 9 (cont.)

Author(Year)	Case confirmation method	Diagnosis period	Lung cancer classification	Control matching	Period of exposure or employment
Cohort studies (n = 7)					
Markowitz(2013)[18]	By chest radiographs	1981 and 1983	ICD-9 code 162 (1981-1998) and ICD-10 codes C-33 and C-34 (1999-2008)	Not reported	1982-2008
Wang (2013)[36]	By pathology or biopsy	The first two decades	The Chinese Radiographic Diagnosis Criteria of Pneumoconiosis	Not reported	1981-2006

*ICD stands for International Classification of Diseases

Table 10 Effect of the Exposure to Asbestos (A) and/or Cigarette Smoking (S) on Lung Cancer Risk

Groups	No. of studies	ORs and RRs* (95% CI)			
		Reference**	A	S	A and S
Case-control studies					
Geographic area					
USA	2	1.00	**1.60** (0.99-2.59)	**3.89** (2.58-5.86)	**6.19** (4.01-9.54)
Europe	7	1.00	**1.71** (1.15-2.54)	**5.63** (2.49-12.71)	**8.89** (4.77-16.56)
Study design					
Population Based	6	1.00	**1.83** (1.32-2.55)	**7.60** (4.09-14.11)	**10.92** (6.54-18.22)
Hospital Based	4	1.00	**1.49** (0.97-2.29)	**3.60** (1.94-6.69)	**6.19** (3.47-11.06)
Cohort studies					
Asbestos type					
Chrysotile	3	1.00	**2.58** (1.13-5.89)	**3.58** (1.75-7.33)	**5.04** (2.50-10.18)
Not reported	3	1.00	**3.05** (1.53-6.08)	**7.33**(4.18-12.85)	**10.47** (7.90-13.88)

*Odds ratios is for case-control, relative risk is for cohort study

** Reference is equal one as control group

Table 11 Effect of the Exposure to Asbestos (A) and/or Cigarette Smoking (S) on Lung Cancer Risk in Case-Control Studies, Stratified by smoking levels

Smoking level	No. of studies	ORs (95% CI)		
		A	S	A and S
Non smokers	2[10,81]	**2.63** (1.43-4.83)	-	-
1-19 cigarettes/day	2	-	**9.98** (3.44-28.96)	**15.38** (7.34-32.24)
>20 cigarettes/day	2	-	**25.41** (8.96-72.00)	**30.31** (15.77-58.25)
0-9 cigarettes/day	3[19,72,76]	**2.63** (1.57-4.42)	-	-
10-19 cigarettes/day	3	-	**8.54** (2.76-14.76)	**13.13** (7.34-32.24)
>20 cigarettes/day	3	-	**15.76** (4.36-56.94)	**25.94** (11.94-56.39)

Table 12 Synergy and Multiplicative Indices between Asbestos Exposure and Cigarette Smoking

Overall risk estimates	**Reference**	**Asbestos**	**Smoking**	**Asbestos and smoking**	**Interaction index***	
					synergy	**multiplicative**
Odds Ratio	1.00	**1.70**(1.31-2.21)	**5.65**(3.38-9.42)	**8.70**(5.78-13.10)	**1.44** (1.26-1.77)	**0.91**(0.63-1.30)
Relative Risk	1.00	**2.72**(1.67-4.40)	**6.42**(4.23-9.75	**8.90**(6.01-13.18)	**1.11** (1.00-1.28)	**0.51**(0.31-0.85)

* Rothman synergy index

Quantitative Synthesis

(i) Case-control studies: A random-effects meta-analysis of 10 studies [11, 19, 21, 72, 73, 75, 76, 77, 78, 79] revealed associations between asbestos exposure and/or smoking, and developing lung cancer. The summary odds ratio of (A+S-) workers compared with (A-S-) workers was 1.70 (95% CI = 1.31-2.21). The summary odds ratio of (A-S+) workers compared with (A-S-) was 5.65 (95% CI = 3.38-9.42). Additionally, the summary odds ratio of (A+S+) workers compared with (A-S-) workers was 8.70 (95% CI = 5.78-13.10). Evidence of heterogeneity was found in A-S+/A-S- and A+S+/A-S- groups (I^2 = 90.6%, p = 0.000 and I^2 = 78.7%, p = 0.000), subsequently (Figure 10). Such heterogeneity probably arises from the differing interaction effects across varying levels of smoking exposure. As shown in Table 8, the results of subgroup analyses according to different characteristics are in close agreement with our major findings.

Publication bias: Begg's funnel plot and Egger's test were performed to assess publication bias of the literature. Publication bias for (i) A+S- was p = 0.437 (Begg's test), and 0.659 (Egger's), (ii) A-S+ was p= 0.252 (Begg's test), and 0.362 (Egger's), and (iii) A+S+, p=0.154 (Begg's test) and 0.294 (Egger's test). Funnel plots suggested evidence of publication bias. There was asymmetry of funnel plots accordant with high heterogeneity studies (A-S+ and A+S+). However, trim and fill analysis showed that the overall odds ratios were unchanged (data shown in supplement).

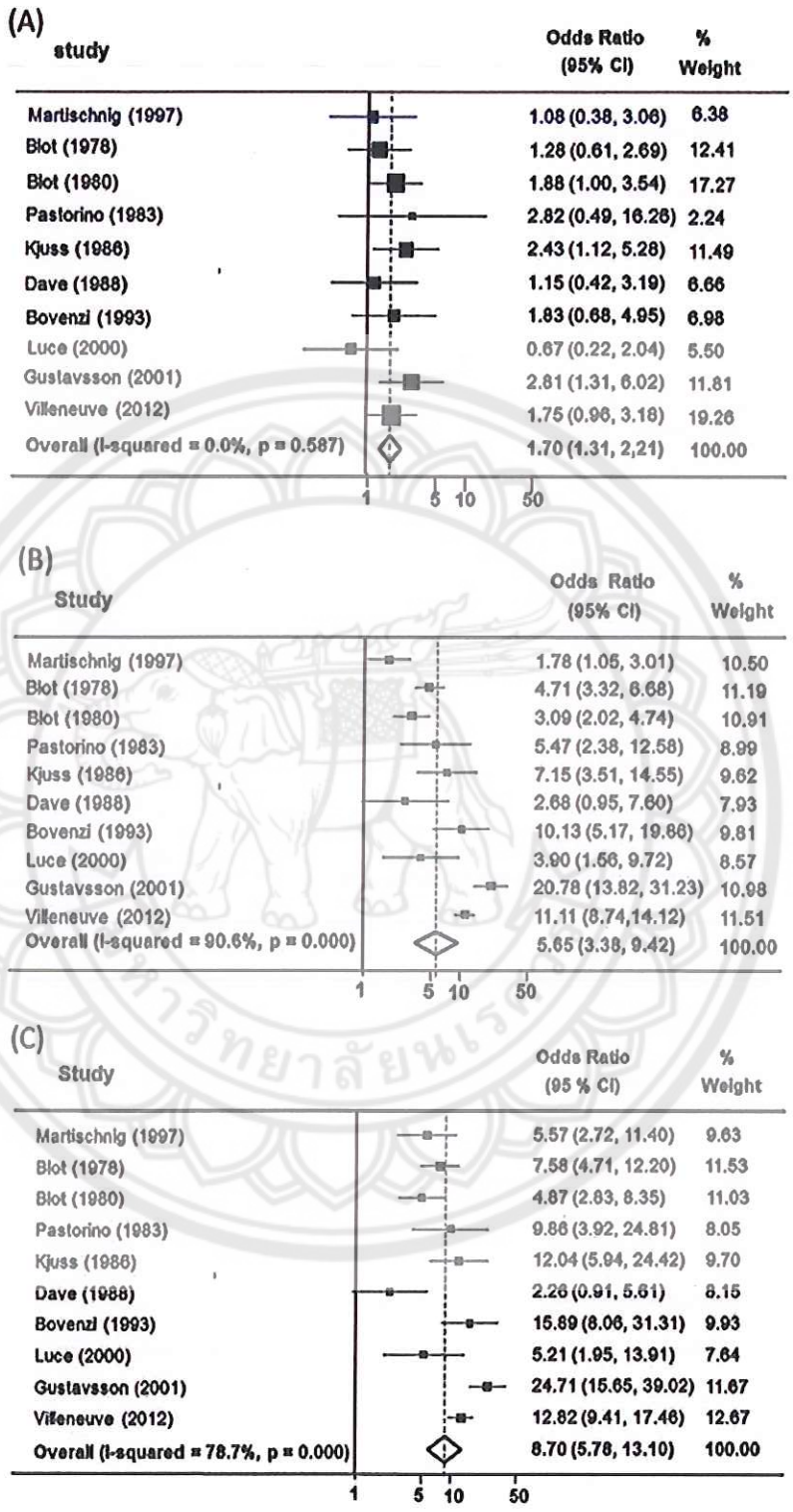


Figure 10 Random-effects meta-analysis of the synergistic effect between asbestos exposure and smoking cause lung cancer- Case control study

(A) Summary odds ratio of asbestos-exposed and non-smoking (A+S-) compared with not asbestos-exposed and non-smoking (A-S-). (B) Summary odds ratio of non-exposure to asbestos and smoking (A-S+) compared with not asbestos-exposed and non-smoking (A-S-). (C) Summary odds ratio of asbestos-exposed and smoking (A+S+) compared with not asbestos-exposed and non-smoking (A-S-)

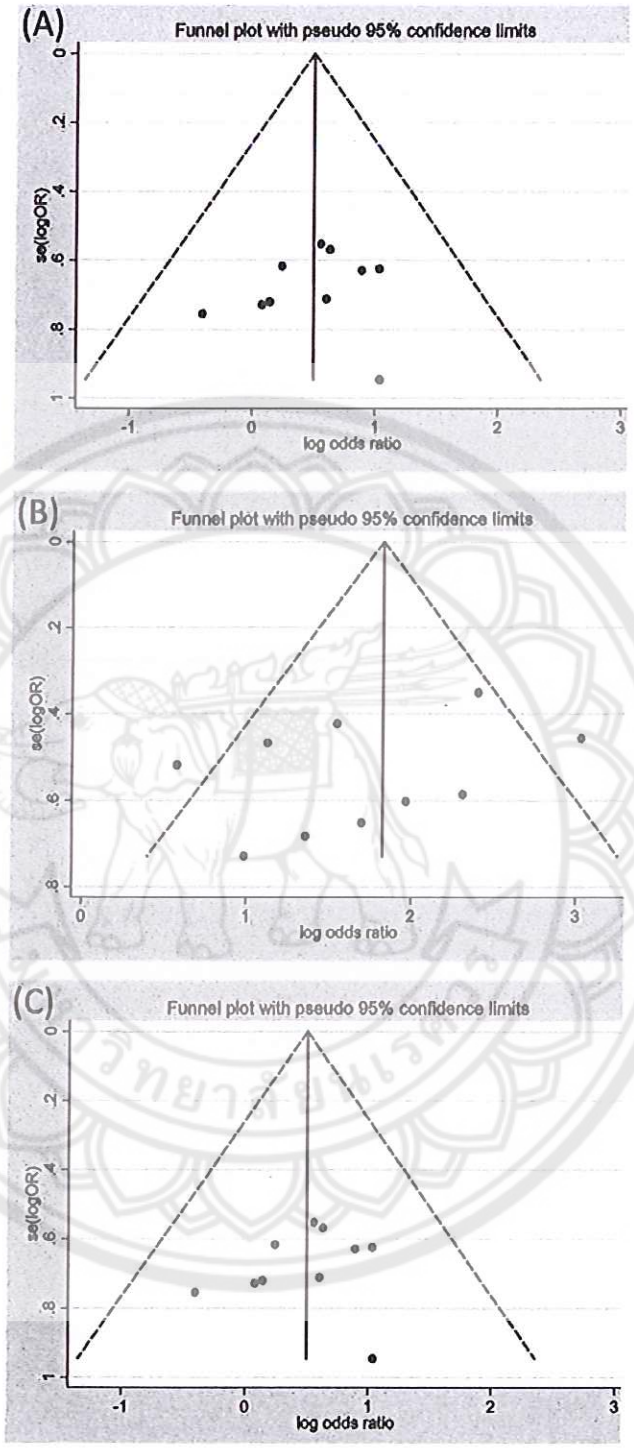


Figure 11 Funnel plot for 10 case-control studies of relationship between asbestos and cigarette smoking on lung cancer with subjects whom are exposed to asbestos and non-smokers (A), subjects whom are not exposed to asbestos and smokers (B) and subjects whom are exposed to asbestos and smokers (C)

(ii) Cohort studies; Seven studies [10, 18, 20, 36, 74, 80, 81] were included in our primary analysis (Figure 12). The summary relative risks for lung cancer in the cohort studies of (A+S-) workers were 2.72 (95% CI = 1.67-4.40), (A-S+) workers were 6.42 (95% CI = 4.23-9.75), and for (A+S+) workers were 8.90 (95% CI = 6.01-13.18) compared with (A-S-) workers. The results of the cohort studies are consistent with the analysis of the case-control studies. Evidence of heterogeneity was not found in cohort studies (I^2 = 0.0 %, p = 0.968, I^2= 25.1%, p = 0.237 and I^2 = 17.3%, p = 0.298).

Publication bias: Evaluation of publication bias for A+S-, A-S+ and A+S+ are Begg's test (p =0.063) Egger's test (p =0.079), Begg's test (p = 0.026) Egger's test (p = 0.065) and Begg's test (p =0.118) Egger's test (p =0.254), respectively. These results did not indicate a potential for publication bias when using funnel plots (data shown in supplement). In addition, case-control studies estimates of the combined effect of asbestos and smoking on lung cancer risk were in concordance with those from cohort studies.

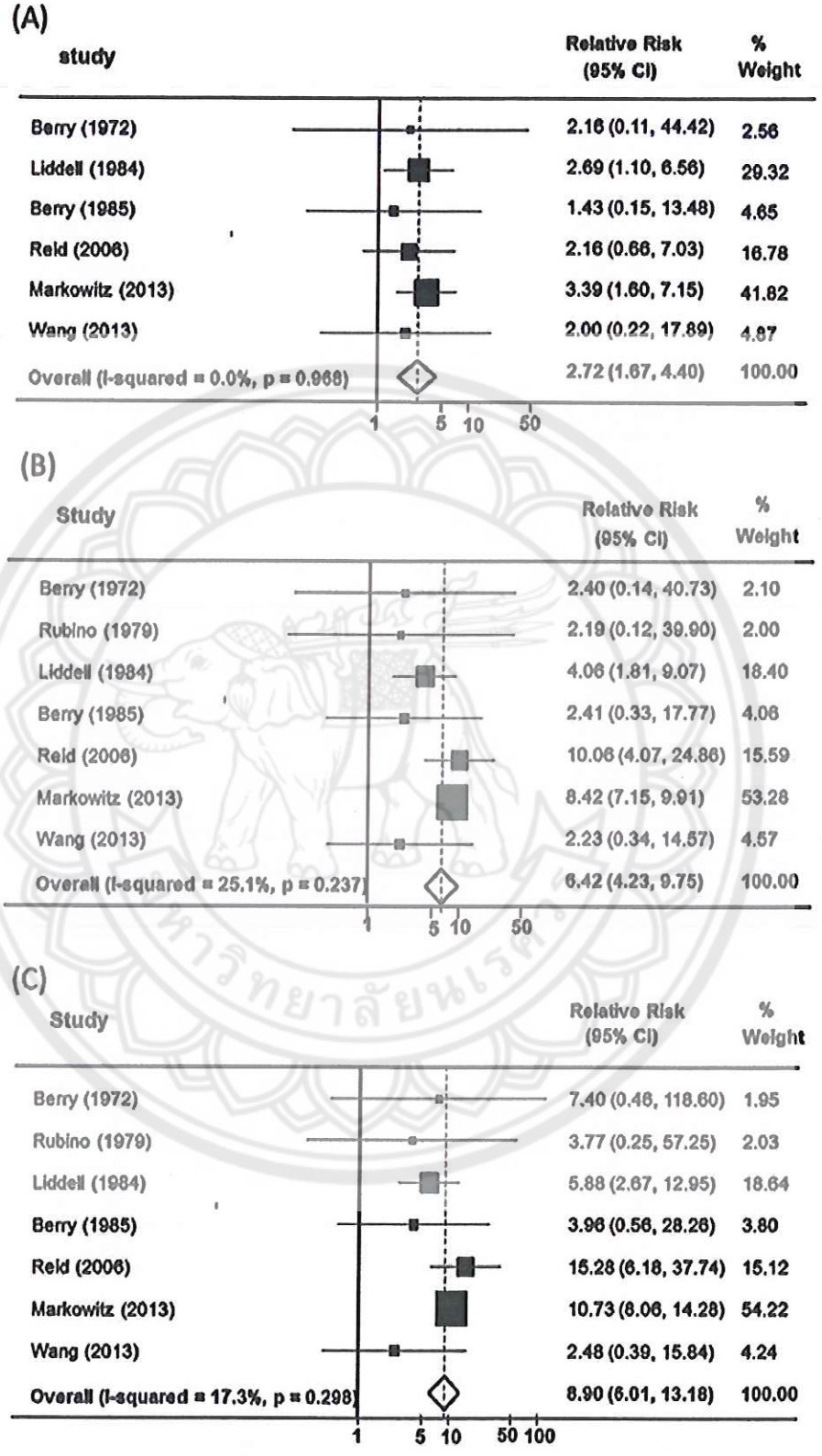


Figure 12 Random-effects meta-analysis of the synergistic effect between asbestos exposure and smoking cause lung cancer- Cohort study

(A) Summary relative risk of asbestos-exposed and non-smoking (A+S-) compared with not asbestos-exposed and non-smoking (A-S-). (B) Summary relative risk of non-exposure to asbestos and smoking (A-S+) compared with not asbestos-exposed and non-smoking (A-S-). (C) Summary relative risk of asbestos-exposed and smoking (A+S+) compared with not asbestos-exposed and non-smoking (A-S-)

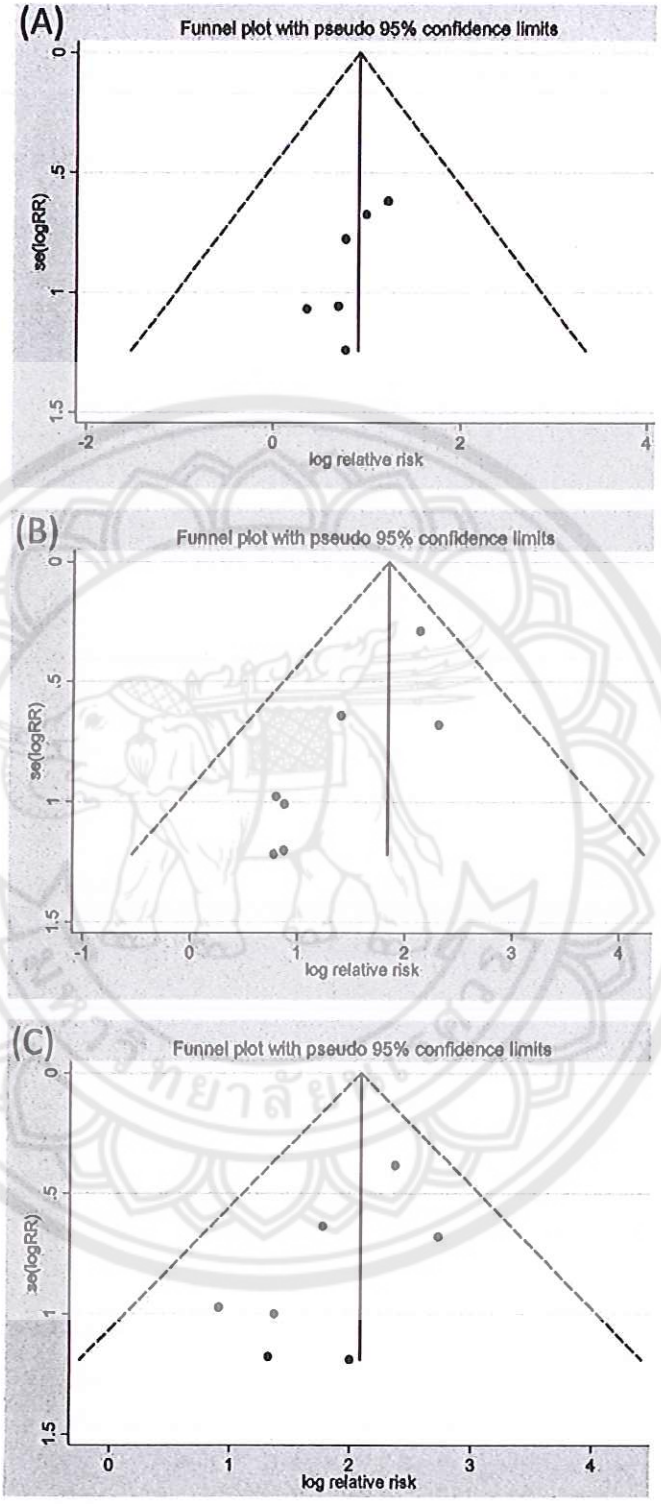


Figure 13 Funnel plot for 7 cohort studies of relationship between asbestos and cigarette smoking on lung cancer with subjects whom are exposed to asbestos and non-smokers (A), subjects whom are not exposed to asbestos and smokers (B) and subjects whom are exposed to asbestos and smokers (C)

Interaction between asbestos exposure and cigarette smoking

Evaluation of interaction is summarized in Table 12. All 17 studies provided data which enabled evaluation of the joint effects of co-exposure of both asbestos and cigarette smoking on the risk of lung cancer. For case-control studies, the interaction index of synergy (*S*) and multiplicative index (*V*) were 1.44 (95% CI = 1.26-1.77) and 0.91 (95% CI = 0.63-1.30), respectively, with corresponding values for the cohort studies of 1.11 (95% CI = 1.00-1.28) and 0.51 (95% = 0.31-0.85). These results suggest that the interaction between asbestos exposure and smoking can be a positive interaction on the additive scale (an additive synergistic effect). There was a suggestion of a negative multiplicative interaction for both case-control and cohort studies. Notably our results do not show a multiplicative effect between the two known human carcinogens.

Discussion

Our results demonstrate a positive synergistic interaction on an additive scale between asbestos exposure and cigarette smoking in workers developing lung cancer (Table 12). Employees exposed to asbestos and having a history of smoking have a higher risk of developing lung cancer than those only exposed to one risk (either smoking or asbestos alone). In contrast, the multiplicative index for case-control studies was close to 1.0, although for cohort studies, a negative multiplication interaction is suggested (*V*=0.51, 95%CI=0.31-0.85).

Some data suggests that smoking does not enhance mesothelioma [82], which implies that the synergistic lung cancer risk arises from the two carcinogens interacting in the same lung tissue. There are several mediators contributing to cigarette smoke and asbestos-induced lung diseases. Both smoking [83] and asbestos [49] elicit chronic inflammation, which is central to tumorigenesis and is augmented through reduced active immunity, increased infections, and compromised tumor surveillance [84, 85]. Tobacco smoke causes inflammation through a vast array of chemical and particulate irritants. Mineral fibers are inflammatory primarily through activation of Nod-like receptor-family protein 3 (NLRP3) of inflammasomes in tissue macrophages. Asbestos fibers evoke vain attacks by macrophages ensuring their continual activation while also adversely affecting function of other immune cells [51,

86]. Symptoms of inflammation include oxidative stress, which is worse in blue asbestos (amosite, crocidolite, tremolite) containing Fe^{2+} ions which generate additional reactive species through Fenton catalysis [87]. The prolonged bio-persistence of these amphiboles further contributes to their greater carcinogenicity than chrysotile and other mineral fibers. Tobacco smoke also contains multiple carcinogens (e.g., 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone or NNK, 1,3-butadiene, ethylene oxide, chromium, polonium-210, arsenic, ethyl carbamate, and hydrazine) that directly interact with DNA [88]. Thus, the common localized inflammatory actions of tobacco smoke and asbestos readily explains additive effects, while the additional actions (direct carcinogenesis and Fenton catalysis) of each insult could account for the additive synergistic interaction.

The present study has some limitations which are mostly inherent in this type of study.

Odds ratios were roughly estimated from the included studies where the measurement methods used and exposure classification varied between studies. For example there were several studies claiming that the duration of asbestos exposure was the same as the period of employment in the workplace. Therefore, short duration jobs reduce the validity and reliability of questionnaires about occupational history. Some studies [76, 77, 79] did not provide estimates of adjusted risks (age, sex, etc.).The methods used to quantitate exposures to asbestos and cigarette smoke were arbitrary and varied across studies. The type of asbestos used was usually not stated. The diagnosis for lung cancer used different criteria (by physician, chest x-ray, radiography, or information taken from the death certificate). In contrast, other studies have objective exposure and clinical criteria (e.g., Markowitz, et al. [18]).The type of lung cancer was rarely stated or even whether mesothelioma was excluded but mesothelioma was never explicitly included. Some case-control studies [72, 78] used control populations who had other diseases (e.g., myocardial infarction, bladder cancer, other malignant neoplasms or other lung disease). Most of these diseases are also smoking-related. Nevertheless, all case-control studies endeavored to match controls for confounders. Some studies have data derived from recalling events that took place 10 years or more before the interview/questionnaire, which raises the issue of recall bias and misclassification. Subgroup analysis by smoking level retained high

heterogeneity (Table 9) probably due to different methods of data collection and measurement, uncertain duration of smoking (only daily number of cigarettes smoked quoted).

Nevertheless, our study has some strength. It includes new data and the selection criteria complied with the PRISMA and MOOSE guidelines to perform the first systematic review and meta-analysis. Our analysis differed from previous analyses because (i), the strict selection criteria and heterogeneity testing, (ii) testing for statistical interaction (additive and multiplicative). Most studies randomly enrolled greater numbers of control subjects from hospital registers or health authority databases thus reducing selection bias. One study [78] excluded participants who provided incomplete questionnaire data, were non-responders, or who had emigrated from the area. These unavoidable variations in the study population and diverse methods utilized readily explain the substantial heterogeneity we detected. While the most dangerous asbestos types are no longer used, other siliceous fibers and chrysotile (in developing nations) are still incorporated into many building products without clear long-term health assessments in humans. Workers exposed to chrysotile showed increased risk of lung cancer (Table 10) [24]. The scientific rigor of cohort studies has improved since the early asbestos work. However, the long latencies for asbestos-induced neoplasms [39] make retrospective study the only practical protocol. Cigarette smoke inhalation and hence airway exposure can be accurately assessed (cigarette numbers, inhalation, filters). However, our study reiterates the difficulty in accurately assessing actual airway exposure to asbestos and was best assessed in the Markowitz, et al. study. [18] Personal monitors provided the best indication of exposure but ultimately, only random sputum fiber counts by public health agencies can provide unbiased and accurate measures of exposure. Another problem highlighted by Markowitz, et al. [18] and our study is accurately diagnosing the end-stage pathology. Again, monitoring by independent public health authorities is the mechanism most likely to yield accurate reporting. In addition, potential confounders including life-style and especially local air quality data need collecting for the same cohorts.

Study2: Smoking increased lung cancer risk in asbestos workers

The objective of this study was to demonstrate asbestos exposure or cigarette smoke can adversely effect on lung cancer. Thus, the study was determined the comparator of asbestos exposure and smoking on lung cancer risk. The subjects are divided into 4 groups as following: (i) asbestos-exposed and smokers (A+S+; control group); (ii) asbestos-exposed and non-smokers (A+S); (iii) no asbestos-exposed and smokers (A-S+); and (iv) non asbestos-exposed and non-smokers (A-S-). An aim is to summaries the overall association between cigarette smoking and asbestos exposure to reflect estimates of lung cancer risk. Either cigarette smoking or asbestos exposure is more enhanced on lung cancer development.

Quantitative Synthesis

Case-control studies

Ten case-control studies [11, 19, 21, 72, 73, 75, 76, 77, 78, 79] were included in our further statistical analysis. All studies demonstrated the association between exposure to asbestos and to smoking and lung cancer (Figure 14). Based on a random-effect meta-analysis, our results show that the exposure to asbestos and smoking are significantly associated with increased risk of lung cancer. Overall odd ratios of the case-control studies of asbestos-exposed and smoking workers compared with non-exposed asbestos and non-smoking workers are 8.70 (95% CI = 5.78-13.10). Overall odd ratios of the case-control studies of asbestos-exposed and smoking workers compared with exposed asbestos and non-smoking workers are 5.17 (95% CI = 3.66-7.31). In addition, overall odd ratios of the case-control studies of asbestos-exposed and smoking workers compared with non-exposed asbestos and smoking workers are 1.45 (95% CI = 1.23-1.72). Evaluation of publication bias for A+S-, A-S+ and A+S+ are Begg's test (p =0.063) Egger's test (p =0.079), Begg's test (p = 0.026) Egger's test (p = 0.065) and Begg's test (p =0.118) Egger's test (p =0.254), respectively. These results did not indicate a potential for publication bias when using funnel plots There was no publication bias found as assessed using funnel plots, Begg's test (p = 0.627) and Egger's test (p = 0.341).

Cohort studies

Seven studies [10, 18, 20, 36, 74, 80, 81] were included in our primary analysis (Figure 11). Relative risk of the cohort studies of asbestos-exposed and smoking workers compared with non-exposed asbestos and non-smoking workers is 8.90 (95% CI = 6.01-13.18). Overall relative risk of asbestos-exposed and smoking workers compared with asbestos-exposed and non-smoking workers is 3.00 (95% CI = 1.69-4.59). Additionally, overall relative risk of asbestos-exposed and smoking workers compared with non-exposed asbestos and smoking workers is 1.45 (95% CI = 1.23-1.73). No evidence of publication bias was observed from the five cohort studies, using Begg's test (p=0.414), Egger's test (p=0.483) and a funnel plot (data not shown). Publication bias: Begg's funnel plot and Egger's test were performed to assess publication bias of the literature. Publication bias for (i) A+S- was p = 0.437 (Begg's test), and 0.659 (Egger's), (ii) A-S+ was p= 0.252 (Begg's test), and 0.362 (Egger's), and (iii) A+S+, p=0.154 (Begg's test) and 0.294 (Egger's test). Funnel plots suggested evidence of publication bias. There was asymmetry of funnel plots accordant with high heterogeneity studies (A-S+ and A+S+). However, trim and fill analysis showed that the overall odds ratios were unchanged.

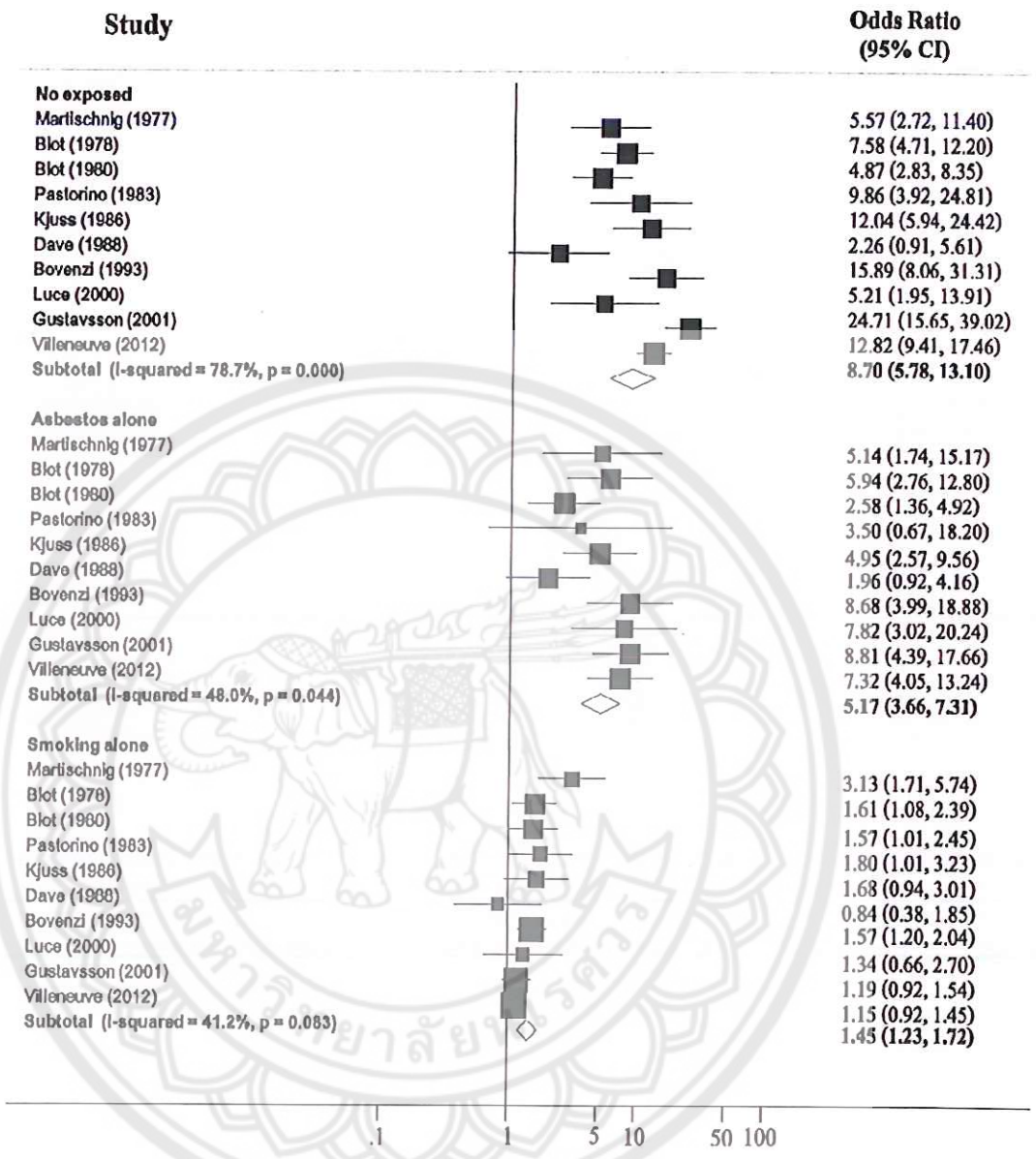


Figure 14 Random-effects meta-analysis of the synergistic effect between asbestos exposure and smoking cause lung cancer- Case control study

(Upper) Summary odds ratios of non-exposure to asbestos-exposed and non-smoking (A-S-) compared with asbestos-exposed and smoking (A+S+). (Middle) Summary relative risk of asbestos-exposed and non-smoking (A+S-) compared with asbestos-exposed and smoking (A+S+). (Lower) Summary odds ratio of non-exposure to asbestos and smoking (A-S+).compared with asbestos-exposed and smoking (A+S+)

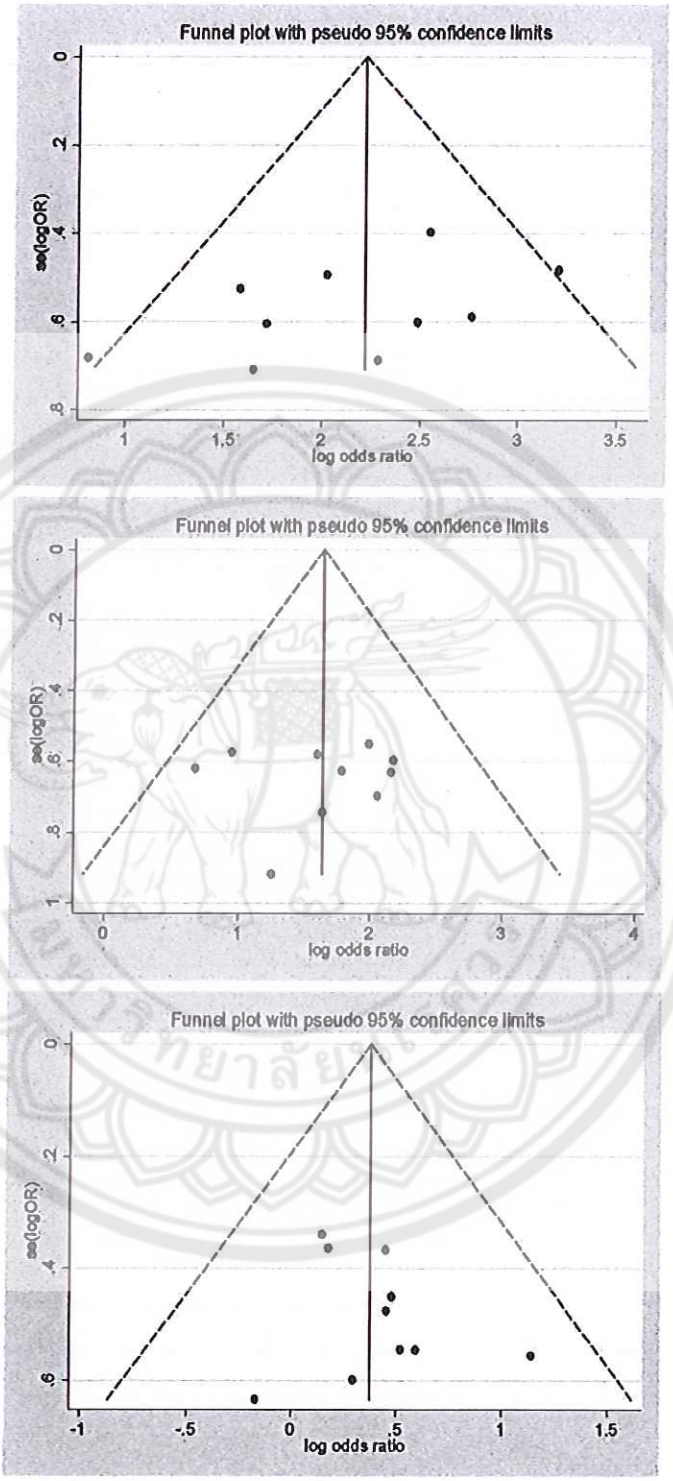


Figure 15 Funnel plot for 10 case-control studies of relationship between asbestos and cigarette smoking on lung cancer with subjects whom are not exposed to asbestos and non-smokers (Upper), subjects whom are exposed to asbestos and non-smokers (Middle) and subjects whom are not exposed to asbestos and smokers (Lower)

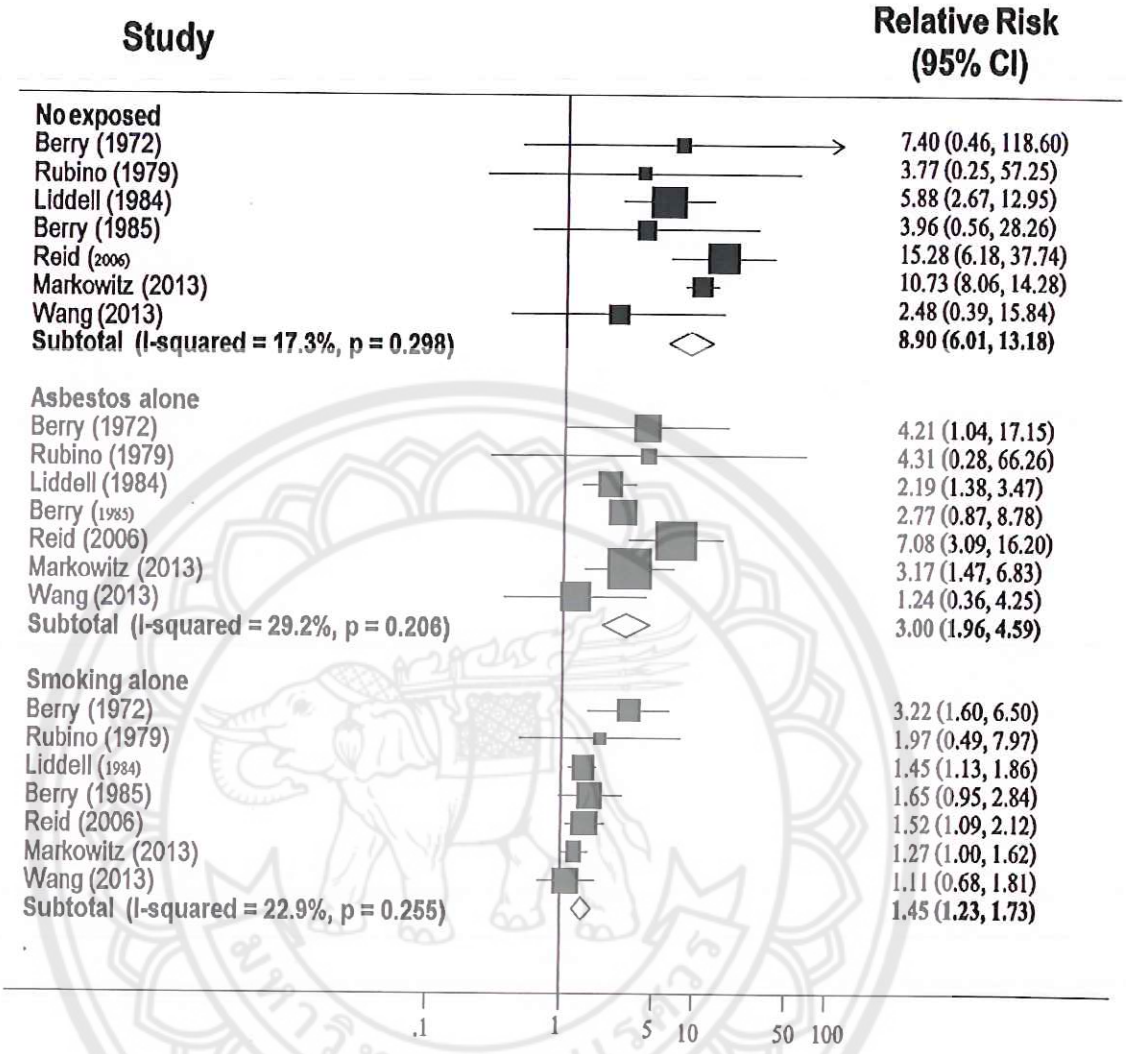


Figure 16 Random-effects meta-analysis of the synergistic effect between asbestos exposure and smoking cause lung cancer- Cohort study

(Upper) Summary relative risk of non-exposure to asbestos-exposed and non-smoking (A-S-) compared with asbestos-exposed and smoking (A+S+). (Middle) Summary relative risk of asbestos-exposed and non-smoking (A+S-) compared with asbestos-exposed and smoking (A+S+). (Lower) Summary odds ratio of non-exposure to asbestos and smoking (A-S+).compared with asbestos-exposed and smoking (A+S+)

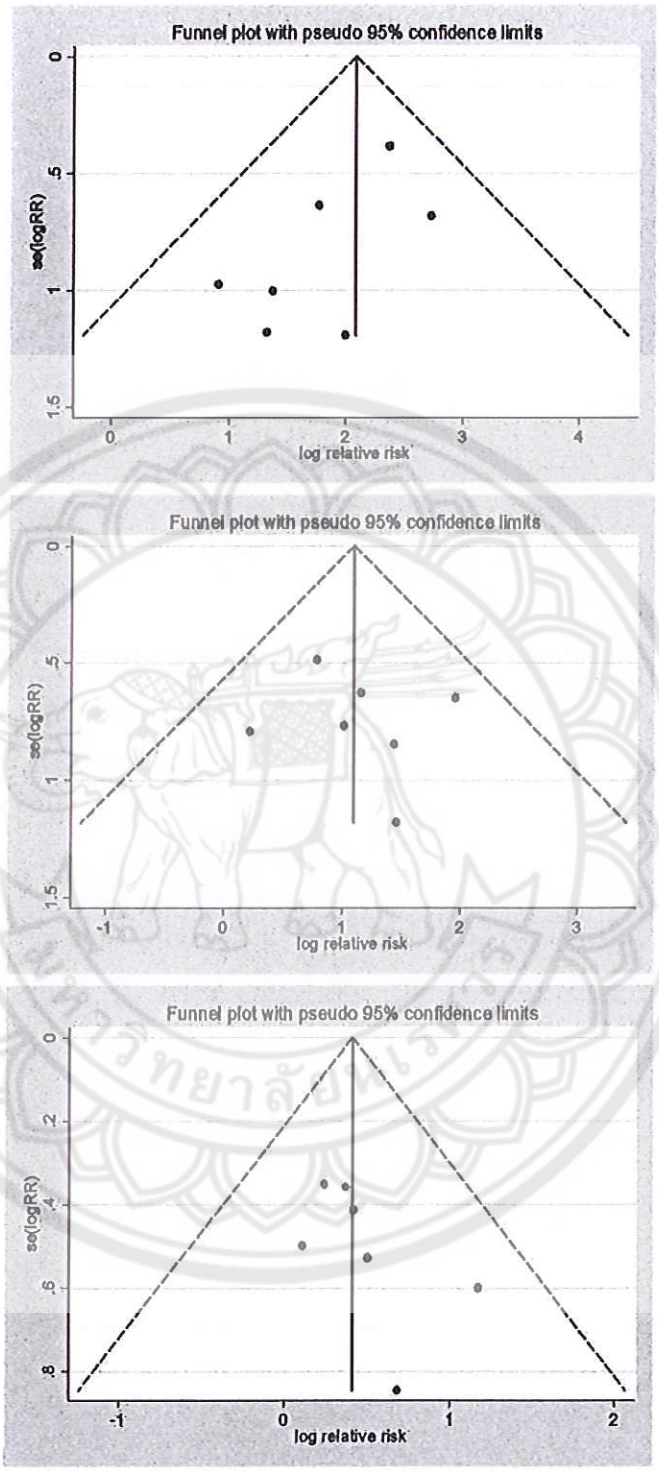


Figure 17 Funnel plot for 7 cohort studies of relationship between asbestos and cigarette smoking on lung cancer with subjects whom are not exposed to asbestos and non-smokers (Upper), subjects whom are exposed to asbestos and non-smokers (Middle) and subjects whom are not exposed to asbestos and smokers (Lower)

Discussion

The results show that cigarette smoking increased risk of lung cancer when compared with asbestos exposure alone. Those workers who both cigarette smoke and have been exposed to asbestos have the highest risk of lung cancer. Smoking is an important risk factor for lung cancer while asbestos exposure is co-factor for inducing the lung disease. Cigarette smoking does not induce risk of malignant mesothelioma in asbestos workers although it was a one factor causing of lung cancer. Thus, all observational studies were included co-exposure of asbestos and smoking associated with lung cancer for quantitative analysis.

In this meta-analysis was shown some limitations. Most studies did not reveal the stage of lung cancers or the subtypes, which might also be source of the heterogeneity. Other confounding variable were also found in smoker groups such as examining method to provide classification of smoking. The number of cigarettes smoked per day or pack-years was also an important factor for smokers over the longest period of time. Moreover, a measurement of asbestos was differently used occupational titles by questionnaire/interview and fiber analysis techniques. These can be performed misclassification bias.

It was a document claimed that chrysotile is safer than amphibole. However, the results of subgroup analysis shown that chrysotile exposure was increased risk of lung cancer in asbestos workers (Table 5). Therefore, these results supported that an adequately reporting was revealed chrysotile exposure causing of health problems.

This meta-analysis also is adequately considered in formulating policies in Thailand and developing countries where continue used asbestos for regulation and banning of asbestos. An evaluation of epidemiologic knowledge of exposure to asbestos is more valuable of human history.

CHAPTER V

CONCLUSION

Conclusion

Study 1: An interaction of asbestos and smoking

The present meta-analysis collected and synthesized data currently available and revealed a positive interaction on an additive scale between asbestos exposure and smoking, while showing little evidence of an interaction on a multiplicative scale. The combined effect of asbestos exposure with moderate and heavy smoking in lung cancer suggested a strongly positive interaction on an additive scale.

Study2: Smoking increased lung cancer risk in asbestos workers

The workers who exposed asbestos and smoked had a highest risk of lung cancer. Cigarette smoking is the most important risk factor for lung cancer in those workers.

REFERENCES

REFERENCES

[1] Siegel, R., Ma, J., Zou, Z. and Jemal, A. (2014). Cancer Statistics, 2014. **CA: A Cancer Journal for Clinicians**, 64, 9-29.

[2] IARC. (2004). Tobacco Smoke and Involuntary Smoking. **Monographs on the Evaluation of Carcinogenic Risks to Humans** (vol.83). Lyon, France: IARC.

[3] Gandini, S., Botteri, E., Iodice, S., Boniol, M., Lowenfels, A., Maisonneuve, P., et al. (2008). Tobacco smoking and cancer: A meta-analysis. **International Journal of Cancer**, 122(1), 155-164.

[4] Marie, N., Freeman, M. K., Thomas, D. F., Margaret, R., Laura, D.-L., Blake, T., et al. (2014). Smoking Prevalence and Cigarette Consumption in 187 Countries. **JAMA**, 311(2), 183-192.

[5] Yang, M. (2011). A current global view of environmental and occupational cancers. **Journal of environmental science and health Part C Environmental carcinogenesis and ecotoxicology reviews**, 29(3), 223-249.

[6] Hubaux, R., Becker-Santos, D. D., Enfield, K. S., Lam, S., Lam, W. L. and Martinez, V. D. (2012). **Arsenic, asbestos and radon: Emerging players in lung tumorigenesis.** Retrieved June 1, 2015, from http://www.ncbi.nlm.nih.gov/pubmed/23173984

[7] IARC. (1977). Asbestos. **IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans**, 14, 1-106.

[8] IARC. (2012). Asbestos (Chrysotile, Amosite,Crocidolite, Tremolite, Actinolite and Anthophyllite). **Monographs on the evaluation of carcinogenic risks to humans** (vol.100). Lyon, France: IARC.

[9] Eastman, A., Mossman, B. and Bresnick, E. (1983). Influence of asbestos on the uptake of benzo(a)pyrene and DNA alkylation in hamster tracheal epithelial cells. **Cancer Research**, 43(3), 1251-1255.

[10] Berry, G., Newhouse, M. and Antonis, P. (1985). Combined effect of asbestos and smoking on mortality from lung cancer and mesothelioma in factory workers. **British Journal of Industrial Medicine**, 42(1), 12-18.

[11] Blot, WJ., Harrington, JM., Toledo, A., Hoover, R., Heath, CW. Jr. and Jr., F. J. (1978). Lung cancer after employment in shipyards during World War II. **New England Journal of Medicine**, 299(12), 620-624.

[12] Lemen, R. A., Dement, J. M. and Wagoner, J. K. (1980). Epidemiology of asbestos-related diseases. **Environmental Health Perspectives**, 34, 1-11.

[13] Tagnon, I., Blot, W. J., Stroube, R. B., Day, N. E., Morris, L. E., Peace, B. B., et al. (1980). Mesothelioma associated with the shipbuilding industry in coastal Virginia. **Cancer Research**, 40(11), 3875-3879.

[14] Kjellstrom, T. and Smartt, P. (2000). Increased mesothelioma incidence in New Zealand: the asbestos-cancer epidemic has started. **New Zealand Medical Journal**, 113(1122), 485-490.

[15] Robinson, B. M. (2012). Malignant pleural mesothelioma: an epidemiological perspective. **Annals of Cardiothoracic Surgery**, 1(4), 491-496.

[16] Sekhon, H., Wright, J. and Churg, A. (1995). Effects of cigarette smoke and asbestos on airway, vascular and mesothelial cell proliferation. **International Journal of Experimental Pathology**, 76(6), 411-418.

[17] Blot, W. J., Davies, J. E., Brown, L. M., Nordwall, C. W., Buiatti, E., Ng, A., et al. (1982). Occupation and the high risk of lung cancer in Northeast Florida. **Cancer Research**, 50(2), 364-371.

[18] Markowitz, S. B., Levin, S. M., Miller, A. and Morabia, A. (2013). Asbestos, asbestosis, smoking and lung cancer. New findings from the North American insulator cohort. **American Journal of Respiratory and Critical Care Medicine**, 188(1), 90-96.

[19] Gustavsson, P., Nyberg, F., Pershagen, G., Schéele, P., Jakobsson, R. and Plato, N. (2002). Low-dose exposure to asbestos and lung cancer: Dose-response relations and interaction with smoking in a population-based case-referent study in Stockholm, Sweden. **American Journal of Epidemiology**, 155(11), 1016-1022.

[20] Reid, A., de Klerk, N. H., Ambrosini, G. L., Berry, G. and Musk, A. W. (2006). The risk of lung cancer with increasing time since ceasing exposure to asbestos and quitting smoking. **Occupational and Environmental Medicine**, 63(8), 509-512.

[21] Villeneuve, P. J., Parent, M. E., Harris, S. A., Johnson, K. C., Paulse, B., Dewar, R., et al. (2012). **Occupational exposure to asbestos and lung cancer in men: evidence from a population-based case-control study in eight Canadian provinces.** Retrieved June 1, 2015, from http://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-12-595

[22] Frank, A. L. and Joshi, T. K. (2014). The Global Spread of Asbestos. **Annals of Global Health**, 80(4), 257-262.

[23] Leong, S., Zainudin, R., Kazan-Allen, L. and Robinson, B. (2015). Asbestos in Asia. **Respirology**, 20(4), 548-555.

[24] Bernstein, D., Dunnigan, J., Hesterberg, T., Brown, R., Velasco, J. A., Barrera, R., et al. (2013). Health risk of chrysotile revisited. **Critical Reviews in Toxicology**, 43(2), 154-183.

[25] Prazakova, S., Thomas, P., Sandrini, A. and Yates, D. H. (2014). Asbestos and the lung in the 21st century: An update. **Clinical Respiratory Journal**, 8(1), 1-10.

[26] Rake, C., Gilham, C., Hatch, J., Darnton, A., Hodgson, J. and Peto, J. (2009). Occupational, domestic and environmental mesothelioma risks in the British population: A case-control study. **British Journal of Cancer**, 100(7), 1175-1183.

[27] United States, E. P. A. (1986). **United States environmental protection agency: Airborne asbestos health assessment update.** USA: Washington, DC.

[28] Robinson, B. W. S. and Lake, R. A. (2005). Advances in Malignant Mesothelioma. **N Engl J Med**, 353, 1591-1603.

[29] Charbotel, B., Fervers, B. and Droz, J. P. (2014). Occupational exposures in rare cancers: A critical review of the literature. **Crit Rev Oncol Hematol**, 90(2), 99-134.

[30] Subhannachart, P., Dumavibhat, N. and Siriruttanapruk, S. (2012). Asbestos-related diseases in Thailand and review literature. **J Med Assoc Thai**, 95(Suppl 8), S71-76.

[31] Donovan, E. P., Donovan, B. L., McKinley, M. A., Cowan, D. M. and Paustenbach, D. J. (2012). Evaluation of take home (para-occupational) exposure to asbestos and disease: a review of the literature. **Crit Rev Toxicol,** 42(9), 703-731.

[32] Magnani, C., Agudo, A., González, C. A., Andrion, A., Calleja, A., Chellini, E., et al. (2000). Multicentric study on malignant pleural mesothelioma and non-occupational exposure to asbestos. **Br J Cancer**, 83(1), 104-111.

[33] Goswami, E., Craven, V., Dahlstrom, D. L., Alexander, D. and Mowat, F. (2013). Domestic Asbestos Exposure: A Review of Epidemiologic and Exposure Data. **Int J Environ Res Public Health**, 10(11), 5629-5670.

[34] Bernstein, D., Chevalier, J. and Smith, P. (2005). Comparison of Calidria chrysotile asbestos to pure tremolite: final results of the inhalation biopersistence and histopathology examination following short-term exposure. **Inhal Toxicol**, 17(9), 427-449.

[35] Mossman, B., Bignon, J., Corn, M., Seaton, A. and Gee, J. (1990). Asbestos: Scientific developments and implications for public policy. **Science**, 247(4940), 294-301.

[36] Wang, X., Yano, E., Lin, S., Yu, I., Lan, Y., Tse, L., et al. (2013). Cancer mortality in Chinese chrysotile asbestos miners: Exposure-response relationships. **PLoS One**, 8(8), e71899.

[37] McDonald, J., Liddell, F., Gibbs, G., Eyssen, G. and McDonald, A. (1980). Dust exposure and mortality in chrysotile mining. **British Journal of Industrial Medicine**, 37(1), 11-24.

[38] Liu, G., Beri, R., Mueller, A. and Kamp, D. (2010). Molecular mechanisms of asbestos-induced lung epithelial cell apoptosis. **Chem Biol Interact**, 188(2), 309-318.

[39] Kamp, D. (2009). Asbestos-induced lung diseases: an update. **Translational Research**, 153(4), 143-152.

[40] Sekido, Y. (2013). Molecular pathogenesis of malignant mesothelioma. **Carcinogenesis**, 34(7), 1413-1419.

[41] Carbone, M. and Yang, H. (2012). Molecular pathways: Targeting mechanisms of asbestos and erionite carcinogenesis in mesothelioma. **Clin Cancer Res,** 18(3), 598-604.

[42] Zhao, H., Gu, J., Xu, H., Yang, B., Han, Y., Li, L., et al. (2010). Meta-analysis of the relationship between passive smoking population in China and lung cancer. **Zhongguo Fei Ai Za Zhi**, 13(6), 617-623.

[43] Teschke, K., Olshan, A., Daniels, J., Roos, A. D., Parks, C., Schulz, M., et al. (2002). Occupational exposure assessment in case-control studies: Opportunities for improvement. **Occup Environ Med**, 59(9), 575-593.

[44] Warren, G. W. and Singh, A. K. (2013). **Nicotine and lung cancer.** Retrieved June 1, 2015, from http://www.ncbi.nlm.nih.gov/pubmed/23599683

[45] Jemal, A., Bray, F., Center, M. M., Ferlay, J., Ward, E. and Forman, D. (2012). Global Cancer Statistics. **CA: A Cancer Journal for Clinicians**, 65(2), 87-108.

[46] Chen, R. J., Chang, L. W., Lin, P. and Wang, Y. J. (2011). **Epigenetic effects and molecular mechanisms of tumorigenesis induced by cigarette smoke: An overview.** Retrieved June 1, 2015, from http://www.hindawi.com/journals/jo/2011/654931/

[47] IARC. (1986). Tobacco smoking. **IARC momographs on the evaluation of carcinogenic risks to humans**, 38, 1-421.

[48] Manning, C., Vallyathan, V. and Mossman, B. (2002). Diseases caused by asbestos: Mechanisms of injury and disease development. **Int Immunopharmacol**, 2(2-3), 191-200.

[49] Hidenori, M., Megumi, M., Suni, L., Yasumitsu, N., Naoko, K.-T., Hiroaki, H., et al. (2012). **Asbestos-induced cellular and molecular alteration of immunocompetent cells and their relationship with chronic inflammation and carcinogenesis.** Retrieved June 1, 2015, from http://www.hindawi.com/journals/bmri/2012/492608/

[50] A. Churg, J. L. Wright, J. Hobson and Stevens, B. (1992). Effects of cigarette smoke on the clearance of short asbestos fibres from the lung and a comparison with the clearance of long asbestos fibres. **Int J Exp Pathol**, 73(3), 287–297.

[51] Nishimura, Y., Maeda, M., Kumagai-Takei, N., Lee, S., Matsuzaki, H., Wada, Y., et al. (2013). Altered functions of alveolar macrophages and NK cells involved in asbestos-related diseases. **Environmental Health and Preventive Medicine**, 18(3), 198-204.

[52] Nelson, H. and Kelsey, K. (2002). The molecular epidemiology of asbestos and tobacco in lung cancer. **Oncogene**, 21(48), 7284-7288.

[53] Jung, M., Davis, W., Taatjes, D., Churg, A. and Mossman, B. (2000). Asbestos and cigarette smoke cause increased DNA strand breaks and necrosis in bronchiolar epithelial cells in vivo. **Free Radical Biology and Medicine**, 28(8), 1295-1299.

[54] Lee, P. (2001). Relation between exposure to asbestos and smoking jointly and the risk of lung cancer. **Occup Environ Med**, 58(3), 145-153.

[55] Rothman, K. J. (1976). The estimation of synergy or antagonism. **American Journal of Epidemiology**, 103(5), 506-511.

[56] Knol, M. J., VanderWeele, T. J., Groenwold, R. H. H., Klungel, O. H., Rovers, M. M. and Grobbee, D. E. (2011). Estimating measures of interaction on an additive scale for preventive exposures. **Eur J Epidemiol**, 26(6), 433-438.

[57] Sanderson, S., Tatt, I. D. and Higgins, J. P. (2007). Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: A systematic review and annotated bibliography. **International Journal of Epidemiology**, 36(3), 666-676.

[58] DerSimonian, R. and Laird, N. (1986). Meta-analysis in clinical trials. **Controlled Clinical Trials**, 7(3), 177-188.

[59] Higgins, J. P. and Thompson, S. G. (2002). Quantifying heterogeneity in a meta-analysis. **Statistics in Medicine**, 21(11), 1539-1558.

[60] Higgins, J. P., Thompson, S. G., Deeks, J. J. and Altman, D. G. (2003). Measuring inconsistency in meta-analyses. **BMJ**, 327(7414), 557-560.

[61] Wong, O. and Raabe, G. K. (1996). Application of meta-analysis in reviewing occupational cohort studies. **Occupational and Environmental Medicine,** 53(12), 793-800.

[62] Begg, C. B. and Berlin, J. A. (1989). Publication bias and dissemination of clinical research. **Journal of the National Cancer Institute**, 81(2), 107-115.

[63] Egger, M. and Smith, G. D. (1998). Bias in location and selection of studies. **BMJ**, 316(7124), 61-66.

[64] Andersson, T., Alfredsson, L., Källberg, H., Zdravkovic, S. and Ahlbom, A. (2005). Calculating measures of biological interaction. **European Journal of Epidemiology**, 20(7), 575-579.

[65] Hosmer, D. W. and Lemeshow, S. (1992). Confidence interval estimation of interaction. **Epidemiology**, 3(5), 452-456.

[66] Bovenzi, M., Stanta, G., Antiga, G. L., Peruzzo, P. and Cavallieri, F. (1992). Occupation and lung cancer risk in the province of Trieste: A case-control study. **Medicina del Lavoro**, 83(4), 338-348.

[67] Kjuus, H., Skjaerven, R., Langård, S., Lien, J. T. and Aamodt, T. (1986). A case-referent study of lung cancer, occupational exposures and smoking. I. Comparison of title-based and exposure-based occupational information. **Scandinavian Journal of Work, Environment & Health**, 12(3), 193-202.

[68] Kjuus, H., Skjaerven, R., Langård, S., Lien, J. T. and Aamodt, T. (1986). A case-referent study of lung cancer, occupational exposures and smoking. II. Role of asbestos exposure. **Scandinavian Journal of Work, Environment & Health**, 12(3), 203-209.

[69] de Klerk, N. H., Musk, A. W., Armstrong, B. K. and Hobbs, M. S. (1991). Smoking, exposure to crocidolite and the incidence of lung cancer and asbestosis. **British Journal of Industrial Medicine**, 48(6), 412-417.

[70] Morabia, A., Markowitz, S., Garibaldi, K. and Wynder, E. L. (1992). Lung cancer and occupation: Results of a multicentre case-control study. **British Journal of Industrial Medicine**, 49(10), 721-727.

[71] Hilt, B., Langård, S., Lund-Larsen, P. G. and Lien, J. T. (1986). Previous asbestos exposure and smoking habits in the county of Telemark, Norway--a cross-sectional population study. **Scandinavian Journal of Work, Environment & Health**, 12(6), 561-566.

[72] Kjuus, H., Langård, S. and Skjaerven, R. (1986). A case-referent study of lung cancer, occupational exposures and smoking. III. Etiologic fraction of occupational exposures. **Scandinavian Journal of Work, Environment & Health**, 12(3), 210-215.

[73] Bovenzi, M., Stanta, G., Antiga, G. L., Peruzzo, P. and Cavallieri, F. (1993). Occupational exposure and lung cancer risk in a coastal area of northeastern Italy. **International Archives of Occupational and Environmental Health,** 65(1), 35-41.

[74] Liddell, F., Thomas, D., Gibbs, G. and McDonald, J. (1984). Fibre exposure and mortality from pneumoconiosis, respiratory and abdominal malignancies in chrysotile production in Quebec. **Annals of the Academy of Medicine, Singapore**, 13(2 Suppl), 340-344.

[75] Luce, D., Bugel, I., Goldberg, P., Goldberg, M., Salomon, C., Billon-Galland, M., et al. (2000). Environmental exposure to tremolite and respiratory cancer in New Caledonia: A case-control study. **American Journal of Epidemiology,** 151(3), 259-265.

[76] Pastorino, U., Berrino, F., Gervasio, A., Pesenti, V., Riboli, E. and Crosignani, P. (1984). Proportion of lung cancers due to occupational exposure. **International Journal of Cancer**, 33(2), 231-237.

[77] Martischnig, K. M., Newell, D. J., Barnsley, W. C., Cowan, W. K., Feinmann, E. L. and Oliver, E. (1977). Unsuspected exposure to asbestos and bronchogenic carcinoma. **British Medical Journal**, 1(6063), 746-749.

[78] Blot, W., Morris, L., Stroube, R. B., Tagnon, I. and Fraumeni, J. J. (1980). Lung and laryngeal cancers in relation to shipyard employment in coastal Virginia. **The Journal of the National Cancer Institute**, 65(3), 571-575.

[79] Dave, S. K., Edling, C., Jacobsson, P. and Axelson, O. (1988). Occupation, smoking and lung cancer. **British Journal of Industrial Medicine**, 45(11), 790-792.

[80] Rubino, G., Piolatto, G., Newhouse, M., Scansetti, G., Aresini, G. and Murray, R. (1979). Mortality of chrysotile asbestos workers at the Balangero Mine, Northern Italy. **British Journal of Industrial Medicine**, 36(3), 187-194.

[81] Berry, G., Newhouse, M. and Turok, M. (1972). Combined effect of asbestos exposure and smoking on mortality from lung cancer in factory workers. **Lancet**, 2(7775), 476-478.

[82] Muscat, J. E. and Wynder, E. L. (1991). Cigarette smoking, asbestos exposure and malignant mesothelioma. **Cancer Research**, 51(9), 2263-2267.

[83] Athanasios, V., Thomais, V., Konstantinos, F. and Spyridon, L. (2013). Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. **International Journal of Environmental Research and Public Health**, 10(9), 3886-3907.

[84] Lee, J., Taneja, V. and Vassallo, R. (2012). Cigarette smoking and inflammation cellular and molecular mechanisms. **Journal of Dental Research**, 91(2), 142-149.

[85] Hanahan, D. and Weinberg, R. (2011). Hallmarks of cancer: the next generation. **Cell**, 144(5), 646-674.

[86] Donaldson, K., Murphy, F., Duffin, R. and Poland, C. (2010). **Asbestos, carbon nanotubes and the pleural mesothelium: A review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma.** Retrieved June 1, 2015, from http://www.ncbi.nlm.nih.gov/pubmed/20307263

[87] Liu, G., Cheresh, P. and Kamp, D. W. (2013). Molecular basis of asbestos-induced lung disease. **Annual Review of Pathology: Mechanisms of Disease**, 8, 161-187.

[88] Hecht, S. (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. **Nature Reviews Cancer**, 3(10), 733-744.

APPENDIX

APPENDIX

PLOS | ONE

RESEARCH ARTICLE

Additive Synergism between Asbestos and Smoking in Lung Cancer Risk: A Systematic Review and Meta-Analysis

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Abstract

Smoking and asbestos exposure are important risks for lung cancer. Several epidemiological studies have linked asbestos exposure and smoking to lung cancer. To reconcile and unify these results, we conducted a systematic review and meta-analysis to provide a quantitative estimate of the increased risk of lung cancer associated with asbestos exposure and cigarette smoking and to classify their interaction. Five electronic databases were searched from inception to May, 2015 for observational studies on lung cancer. All case-control (N = 10) and cohort (N = 7) studies were included in the analysis. We calculated pooled odds ratios (ORs), relative risks (RRs) and 95% confidence intervals (CIs) using a random-effects model for the association of asbestos exposure and smoking with lung cancer. Lung cancer patients who were not exposed to asbestos and non-smoking (A-S-) were compared with; (i) asbestos-exposed and non-smoking (A+S-), (ii) non-exposure to asbestos and smoking (A-S+), and (iii) asbestos-exposed and smoking (A+S+). Our meta-analysis showed a significant difference in risk of developing lung cancer among asbestos exposed and/or smoking workers compared to controls (A-S-), odds ratios for the disease (*95% CI*) were (i) 1.70 (A+S-, *1.31–2.21*), (ii) 5.65; (A-S+, *3.38–9.42*), (iii) 8.70 (A+S+, *5.8–13.10*). The additive interaction index of synergy was 1.44 (95% CI = *1.26–1.77*) and the multiplicative index = 0.91 (95% CI = *0.63–1.30*). Corresponding values for cohort studies were 1.11 (95% CI = *1.00–1.28*) and 0.51 (95% CI = *0.31–0.85*). Our results point to an additive synergism for lung cancer with co-exposure of asbestos and cigarette smoking. Assessments of industrial health risks should take smoking and other airborne health risks when setting occupational asbestos exposure limits.

Introduction

Lung cancer is responsible for 20% of all global cancer deaths. Its latency period is long (~20 yr) and survival rate poor (10%) [1]. Meta-analyses of epidemiological studies demonstrated that smoking had a strong relationship with lung cancer [2,3] and 70–90% of lung cancer patients are directly attributed to cigarette smoking [4]. Several compounds in tobacco smoke are classified as human carcinogens (Group 1) by the IARC including tobacco specific nitrosamines and benzo(a)pyrene, a carcinogenic polycyclic aromatic hydrocarbon [4,5]. Second-hand smoke also increases the risk of developing lung cancer by an estimated 25% in by-standers [6]. Besides smoking, other risk factors for lung cancer are arsenic, particulates from diesel engine exhausts, radon, and exposure to asbestos and other mineral fibers, [7,8].

Asbestos is a group of naturally occurring silicate mineral fibers widely used in building materials, vehicle brakes and thermal insulators since the 1900s. Asbestos types are classified according to their structures, chemical composition and thermal stability. Chrysotile or white asbestos (mainly $Mg_3(Si_2O_5)(OH)_4$) [9,10] accounts for most current use where asbestos is permitted while amosite (brown) and crocidolite (blue asbestos), belonging to the amphibole class, are stronger, more durable, and more heat resistant than chrysotile. There are many well documented lung disease cases in asbestos factory workers and miners from 1900 onwards [11–15]. The most common asbestos-associated diseases are benign pleural disease, asbestosis, lung carcinoma (small cell, squamous, and adenocarcinoma) and mesothelioma [16]. Mesothelioma has a very high association with asbestos exposure but otherwise uncommon [17]. It has high incidences among males of western countries and Japan where it is projected to peak between 2012 and 2030, a latency of 40–50 years after the peak use of asbestos during the 1930s-1970s [18].

Numerous studies have shown a clear association between carcinogenesis and either smoking or asbestos. However, associations may result from independent and unrelated mechanisms and therefore show additive effects while effects greater than summed individual actions implies biological interactions [19,20]. This is commonly referred to as synergism [21] but additive synergism is more appropriate. Conversely, a smaller effect than the sum of effects may be due to antagonistic interactions. Synergism might, less commonly, be multiplicative due to different types of interaction, for example where an effect requires the activation of two or more serial processes. Such distinctions are important for both possible treatment considerations and public health such as identifying those at greatest risk of disease. Some authors have sought to assess interactions between asbestos and smoking on lung cancer [22,23], and found the effects to be additive [24], more than additive [25] and multiplicative [26,27]. In animal experiments, co-exposure to asbestos and cigarette smoke also found contradictory interaction models [28–30]. Two previous meta-analyses [31,32] found associations between asbestos exposure and smoking for increased lung cancer risk and that the two carcinogenic effects were greater than the sum of their separate actions but again failed to agree on the type of interaction (multiplicative or additive). These reviews had some weakness (assessing individual interactive effects in each study and could not explain the dose-response for asbestos exposure). Also, they have been superseded by additional studies which relate asbestos exposure with smoking and lung cancer [22–27]. Besides increasing the power and weight of the data, these later studies were better designed and controlled, especially the Markowitz et al. study [24], and therefore better able to resolve these issues. Thus, we incorporated this data into a new systematic review and meta-analysis. We anticipate that such a study will better inform the risk assessment process in developing nations where most male semi-skilled workers are smokers, and occupational asbestos exposure continues to pose a health risk in populations where lung disease is a leading cause of mortality [33].

Methods

The study was conducted and reported using the PRISMA (S1 PRISMA Checklist) [34] and MOOSE [35] guidelines.

Search Strategy and study selection

We searched titles and abstracts PubMed, Embase, Scopus, ISI Web of Knowledge, and TOXLINE databases from their inception to May 2015. Combinations of the following key words were used: asbestos, crocidolite, amosite, chrysotile, tremolite, actinolite, anthophyllite, cigarette, cigarette smoke, cigarette smoking, pipe, cigar, tobacco, tobacco smoking, lung cancer, mesothelioma, lung carcinoma, and lung adenocarcinoma. There was no language restriction. Additional studies were also hand-searched from bibliographies of the selected studies.

Inclusion and exclusion criteria

Studies were included if they met all of the following criteria: (1) original articles published in peer-reviewed journals; (2) human studies; (3) observational studies; (4) studies investigating associations between asbestos exposure and smoking with lung cancer, and; (5) studies reporting sufficient data for calculating odds ratios and relative risks. The studies not meeting the inclusion criteria described above were excluded. If there were duplicate populations, only the studies providing the most details, grater number of participants, followed populations for longer follow-up periods, or the most recently published were selected for meta-analysis. Two reviewers (YN, WT) independently appraised titles and abstracts retrieved from the comprehensive searches. The controversial reviews were discussed and resolved by a third reviewer (OL). If further details were required, the reviewers contacted the authors for more information.

Data Abstraction and Quality Assessment

Information extracted from each study included first author, publication year, geographic area, study type (hospital-based case-control, population-based case-control, nested case-control, retrospective cohort, prospective cohort, and cross-sectional), total number of cases, and controls, fiber type (chrysotile, crocidolite, tremolite), industry type, measurement of asbestos and/or smoking exposure, asbestos exposure assessment method, definition of asbestos exposure and/or smoking, period of employment/exposure, measurement method (asbestos exposure, smoking), and classification of outcome. The Newcastle-Ottawa quality assessment scale (NOS) was used to assess the quality of the selected observational studies. The categories of NOS was based on selection of participants, comparability of study groups, and the exposure of interest (case-control studies) or outcome of interest (cohort studies) [36]. When each category is satisfied it attracts one or sometimes two 'star(s)' and a maximum of 9 stars for either case-control or cohort study, indicates the highest quality study [37].

Statistical Analysis

Asbestos exposure was arbitrarily taken as more than 100 air-borne fiber-yr/ml of environmental air for >5% of their work time and cigarette smoking was categorized as smokers who smoked >15 cigarettes/day. Those subjects having lower and shorter fiber exposures and lower cigarette consumption were deemed as non-exposed or non-smokers, respectively.

Using the above cut-offs, subjects were placed into four groups: (1) those people not exposed to asbestos and non-smokers were classified as not exposed to asbestos and non-smoking (A-S-), (2) workers exposed asbestos and non-smokers were classified as asbestos-exposed and non-

smoking (A+S-), (3) those not exposed to asbestos but smoked were grouped as non-exposed to asbestos and were smokers (A-S+), and (4) workers exposed to asbestos and smoked were classified as asbestos-exposed and smokers (A+S+). The primary outcome of the pooled analysis focused on comparing the summary effect of lung cancer risk in people without asbestos exposure and non-smoking versus co-exposure to asbestos and/or smoking as follows: (i) A+S- compared with A-S- (ii) A-S+ compared with A-S-, and (iii) A+S+ compared with A-S- and interaction between asbestos and smoking were evaluated using the Rothman Synergy Index [38]. Summary effect estimates were assessed discretely by averaging the natural logarithmic OR and/or RR weighted by their inverse variances. The pooled effect estimates were calculated using a random effects model by the method of DerSimonian and Laird [39]. Heterogeneity among selected studies was determined using the Q-statistic and *I*-squared tests [40]. *I*-squared (I^2) values of 25%, 50%, and 75% represented low, moderate, and high degrees of heterogeneity, respectively [41]. The meta-analysis of case-control and cohort studies were conducted separately due to differences in the nature of study design [42].

Subgroup analyses were performed according to the geographic area (Europe, America, others), asbestos type, study design (hospital or population, retrospective, prospective), and stratification of smoking level were used to assess the impacts of study characteristics on outcomes. Publication bias was quantified using funnel plot, Begg's test and Egger's test, where $p>0.05$ for both tests was considered to have no significant publication bias [43,44]. All analyses were performed using STATA software V.10.1 (Stata Corp, College Station, TX, USA).

Determination of interactive effect

For measurement of interaction, there are 2 models to calculate this: the additive and the multiplicative scales. If these yield more than additive and multiplicative, there is a positive interaction. If less than additive/multiplicative, it is referred to as a negative interaction. The word "synergistic" means the effect two exposures is greater than the combined effect of each exposure. Thus, the value of interaction is more than either the additive or the multiplicative scales as appropriate, i.e., either additive or multiplicative synergism.

The joint effect of exposure to asbestos and smoking was first examined by estimating odds ratio (ORs) and relative risk (RRs). To determine whether co-exposure to asbestos and smoking is an additive and multiplicative scale, the synergy (S) and multiplicative (V) indices were calculated as follow [38,45].

Synergy index (S)

$$S = \frac{X_{AS} - X_0}{X_A + X_S - 2X_0}$$

Multiplicative index (V)

$$V = \frac{X_0 \times X_{AS}}{X_A \times X_S}$$

Where X_0 is the odds ratio and/or relative risk for lung cancer among non-exposed to asbestos and non-smokers; X_A is the corresponding value for lung cancer among asbestos exposure in non-smokers; X_S is for lung cancer and smoking in those without asbestos-exposure; and X_{AS} is for lung cancer and co-exposure to asbestos and smoking. The synergy index (S) is an interaction on an additive scale. The interpretation is $S = 1$ suggests no interaction between asbestos exposure and smoking on lung cancer; $S > 1$ suggests a positive interaction (synergism); and $S < 1$ suggests a negative interaction (i.e., antagonism). For the multiplicative index (V), it can

be interpreted as either: when $V = 1$, there is no interaction on the multiplicative scale; when $V > 1$, the multiplicative interaction is positive; or when $V < 1$, it is negative. Confidence intervals (CIs) were calculated using the method of Rothman, and Andersson et al. [38,45,46].

Results

Study Selection

We identified 2,499 records of which 2,479 were duplicated, irrelevant, review articles, case reports, non-human or experimental studies, or lacked lung cancer outcomes or lacking control groups, and were excluded. Five additional publications meeting the inclusion criteria were added from the bibliographies of the retrieved articles (Fig 1). In the final review of 25 studies, we excluded 5 studies [47–51] due to duplicate populations, and 3 studies [52–54] had insufficient data. Only one by Kjuus et al [55] was selected of three articles [47,48,55] which analyzed the same data. Case-control studies by Bovenzi (1992 and 1993) [49,56], the cohort studies of McDonald 1980 and Liddell 1984 [51,57]; and cohort studies of Klerk 1991 and Reid 2006 [26,50] also described the same populations of which the most recent [26,56,57] was selected. The Blot et al. study 1982 [52] did not report smoking status in asbestos-exposed populations. Finally, the studies of Hilt et al. 1986, and Markowitz et al. 1992 [53,54] were excluded because numbers of controls were missing. Therefore, a total of 17 studies (10 case-control and 7 cohort studies) were included for meta-analysis. The 13 included studies were identified using the search terms, and another 4 studies derived from their bibliographies.

Study Characteristics

The characteristics and information of the included studies are shown in Table 1. The 10 case-control studies [22,25,27,55,56,58–62], contained 10,223 participants in all of which 4,768 were population-based controls, and 1,128 hospital-based controls. Seven cohort studies [23,24,26,57,63–65] had an aggregate of 64,924 participants, comprising of the 3,316 cases and 61,608 controls. In all the included studies asbestos exposure was occupational. Where

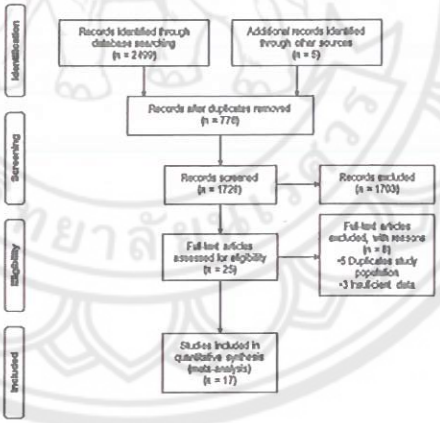

Fig 1. Summary of study search and selection.

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Table 1. Characteristics of studies included in the meta-analysis.

First author(year)	Location	Industrial type*	Asbestos type	Study design	Total population Case (n)	Total population Control (n)	NOS**
Case-control studies (n = 10)							
Martischnig(1977)	United Kingdom	Not specified	Not reported	Hospital-Based	201	201	6
Blot(1978)	Coastal Georgia, USA	Shipbuilding	Not reported	Hospital-Based	458	553	5
Blot(1980)	Coastal Virginia, USA	Shipyard	Not reported	Population-Based	319	341	6
Pastorino(1984)	Lombardy Northern, Italy	Manufacturing, textiles	Mixed	Population-Based	106	226	6
Kjuus(1986)	Southern Norway	Not specified	Not reported	Hospital-Based	176	176	7
Dave(1988)	Southeast Sweden	Not specified	Not reported	Hospital-Based	62	198	5
Bovenzi(1993)	Northeast Italy	Not specified	Not reported	Population-Based	516	561	6
Luce(2000)	New Caledonia, France	Mining & refining	Tremolite	Population-Based	103	110	6
Gustavsson(2002)	Stockholm, Sweden	Not specified	Not reported	Population-Based	768	1519	6
Villeneuve(2012)	8 locations, Canada	Not specified	Not reported	Population-Based	1618	2011	7
Cohort studies (n = 7)							
Berry(1972)	London, England	Asbestos factory	Not reported	Prospective	61	1678	6
Rubino(1979)	Balangero mine, Italy	Mining	Chrysotile	Prospective	12	54	7
Liddell(1984)	Quebec, Canada	Mining & milling	Chrysotile	Prospective	223	715	6
Berry(1985)	London, England	Asbestos factory	Not reported	Prospective	66	1268	6
Reid (2006)	Western Australia	Mining & milling	Crocidolite	Prospective	138	2595	7
Markowitz(2013)	USA	Insulator	Not reported	Prospective	2760	55161	8
Wang (2013)	China	Mining	Chrysotile	Prospective	56	137	7

*All studies are occupational exposures

**NOS = Newcastle Ottawa-Scale

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reported, the average participant age was approximately 60 (range 40–80 y) for case control studies. Some [22,60] reported the type of asbestos used (tremolite or mixed asbestos), while the remaining eight [25,27,55,56,58,59,61,62] did not categorize the asbestos (Table 1). The settings for the exposure was occupational, either asbestos mines (one study [22]), ship building/repair (two studies [59,62]), textile production (one study [60]), and the remaining six [25,27,55,56,58,61] studies failed to specify. Environmental monitoring was measured by using the membrane filter method and were analyzed by phase contrast microscopy [25] but most studies relied on personal/telephone interview and/or questionnaire. Smoking habits of participants were quantified by personal/telephone interview and/or questionnaire. If the subject had already died, the appropriate information was sought from their next-of-kin or spouse (Table 2).

There were seven cohort studies, and all of these collected asbestos exposure data prospectively and also prospectively for smoking data in six studies and retrospectively in one [64].

Table 2. Descriptions of Asbestos Exposure and Smoking of Included Studies.

First author (year)	Measurement of exposure	Definition of asbestos exposure Exposed	Definition of asbestos exposure Non-exposed	Measurement of exposure	Definition of smoking Exposed	Definition of smoking Non-exposed
Case-control studies (n = 10)						
Martischnig (1977)	Questionnaire	Occupational history (work in asbestos manufacturing or used asbestos)	No occupational history	Questionnaire	14 cigarettes/ day or more	0–14 cigarettes/day
Blot(1978)	Personal interview	Occupational history (work in shipbuilding or used asbestos)	No occupational history (never work in shipbuilding)	Personal interview	10 cigarettes/ day or more	<1/2 pack/day and stopped smoking at least 10 years
Blot(1980)	Personal interview	Occupational history (shipyard)	No occupational history (never work in shipyard)	Personal interview	10 cigarettes/ day or more	<1/2 pack/day and stopped smoking at least 10 years
Pastorino (1984)	Personal interview	Exposed to asbestos only	Exposed other carcinogenic chemicals	Personal interview	10 cigarettes/ day or more	0–9 cigarettes/day
Kjuus(1986)	Personal interview and questionnaire	Asbestos exposure at least 1 year or more and job title information	No exposure and no job title	Personal interview and questionnaire	10 cigarettes/ day or more	0–9 cigarettes/day
Dave(1988)	Self-administered questionnaire and telephone interview	Occupational history (works related to asbestos)	Occupational history (other works)	Self-administered questionnaire and telephone interview	>60 cigarette-years	0 cigarette-years
Bovenzi(1993)	Personal interview	Occupational history (classified by job titles and asbestos exposure information)	No occupational history	Personal interview	>1 cigarette/day	No smoked
Luce(2000)	Personal interview	Occupational history (classified by expert assessment)	No occupational history	Personal interview	>20 pack-years	< 20 pack-years
Gustavsson (2002)	Questionnaire, telephone interview and environmental measurement	Occupational history and asbestos exposure > 0 fiber-years	No occupational history and asbestos exposure 0 fiber-years	Questionnaire and telephone interview	>1 cigarette/day	No smoked
Villeneuve (2012)	Questionnaire	Occupational history (classified by concentration, frequency and reliability)	No occupational history	Questionnaire	10 pack-years or more	< 10 pack-years
Cohort studies (n = 7)						
Berry(1972)	Questionnaire	Occupational history	No occupational history	Questionnaire	smoked	No smoked
Rubino(1979)	Environmental measurement	Occupational history (mining)	No occupational history	Personal interview	smoked	No smoked
Liddell(1984)	Environmental measurement	Cumulative exposure >100 fiber/year	Cumulative exposure 0–100 fiber/year	Questionnaire	>1 pack-years	0 pack-years
Berry(1985)	Questionnaire	Occupational history	No occupational history	Questionnaire/ interview	smoked	No smoked
Reid(2006)	Questionnaire and environmental measurement	Occupational history	No occupational history	Questionnaire	Smoked and ex-smoked < 20 years	No smoked and ex-smokers > 20 years

(*Continued*)

Table 2. (*Continued*)

First author (year)	Measurement of exposure	Definition of asbestos exposure Exposed	Definition of asbestos exposure Non-exposed	Measurement of exposure	Definition of smoking Exposed	Definition of smoking Non-exposed
Markowitz (2013)	Clinical method (x-ray and spirometry)	Occupational history (insulation)	No occupational history	Not reported	smoked	No smoked
Wang(2013)	Environmental measurement	Cumulative exposure >20 fiber-year/ml	Cumulative exposure <20 fiber-year/ml	Questionnaire/ interview	smoked	No smoked

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The mean follow-up period of cohort studies was 19.3 yr. Exposure was to chrysotile in three studies [23,57,65], one study to crocidolite [26], and the asbestos type was unspecified in remaining three studies [24,63,64] (Table 1). Four studies [23,26,57,65] were from mining and three studies [24,63,64] originated from factories making asbestos products. Workplace asbestos exposure was assessed by lung histology, counting fibers trapped by midget impingers or membrane filters [23,57,65], a long-duration personal konimeter [26], or postal questionnaires [63,64]. Only one study assessed exposure by chest X-ray radiographs and a low FEV1 by spirometry [24]. Smoking was assessed by interviewing or questionnairing the workers or their next-of-kin (Table 2). Diagnosis of lung cancer was confirmed by histological examination of lung biopsies, chest X-ray, CT scan, MRI, bronchoscopy, or thoracoscopy. Most studies classified lung cancer using the International Classification of Diseases (ICD), published by the World Health Organization (Table 3).

Quality Assessment

The methodological quality of case-control studies was summarized as a mean NOS of 6 (range 5–7) and a score of 6.7 (range 6–8) for cohort studies (Table 1).

Quantitative Synthesis

(i) Case-control studies: A random-effects meta-analysis of 10 studies [22,25,27,55,56,58–62] revealed associations between asbestos exposure and/or smoking, and developing lung cancer. The summary odds ratio of (A+S-) workers compared with (A-S-) workers was 1.70 (95% CI = 1.31–2.21). The summary odds ratio of (A-S+) workers compared with (A-S-) was 5.65 (95% CI = 3.38–9.42). Additionally, the summary odds ratio of (A+S+) workers compared with (A-S-) workers was 8.70 (95% CI = 5.78–13.10). Evidence of heterogeneity was found in A-S+/A-S- and A+S+/A-S- groups (I^2 = 90.6%, p = 0.000 and I^2 = 78.7%, p = 0.000) (Fig 2A–2C). As shown in Table 4, the results of subgroup analyses according to different characteristics are in close agreement with our major findings. Such heterogeneity probably arises from the differing interaction effects across varying levels of smoking exposure. We stratified studies with similar smoking classification by subdivision into 3 levels: non-smokers (non-smoking or light smoking), moderate smokers (1–19 cigarettes/day) and heavy smokers (>20 cigarettes/day) (Table 5). There were no differences between non-smokers 2.63 (95% 1.43–4.83) and light smokers 2.63 (95% 1.57–4.42) for exposed-asbestos group. But for both subgroups, the moderate and heavy smoking categories showed elevated odds ratios with asbestos exposure.

Table 3. Descriptions of Outcome of Included Studies.

Author (Year)	Case confirmation method	Diagnosis period	Lung cancer classification	Control matching	Period of exposure or employment
Case-control studies (n = 10)					
Martischnig (1977)	Radiography, bronchoscopy or thoracotomy	1972–1973	Not reported	Age (±2 years)	1–5 years and 6 years and over
Biot(1978)	By physician	1970–1976	ICD 8 162.1	Sex, race, age (±2 years)	6 months or more
Biot(1980)	By physician	1976	ICD 162.1	Race, age, death year, city of residence	6 months or more
Pastorino (1984)	By physician	1978–1979	Not reported	Age (±2 years)	6 months or more
Kjuus(1986)	By examination of histology		ICD 162–163	Age (±5 years)	1979–1983
Dave(1988)	Not reported	1980–1982	ICD 162–163	Age, sex	Not reported
Bovenzi (1993)	Histology, autopsy reports		ICD 9th 162	Age (±2 years)	Not reported
Luce(2000)	Clinical, radiological & endoscopic	1993–1995	ICD for oncology topography code 160–162,148	Sex, age (±5 year)	Not reported
Gustavsson (2002)	Not reported	1985–1990	ICD 7th 162.1	Age (±5 year) and year of inclusion study (1985–1990)	1969–1973
Villeneuve/ 2012	By examination of histology	1994–1997	ICD 9th 162	Age, sex	At least 12 months
Cohort studies (n = 7)					
Berry(1972)	By examination of histology	Not reported	ICD 162,163	Not reported	Men 1933–1955 Women 1936–1942
Rubino(1979)	By physician	1957	ICD 7 162/163	Age (±1 year)	1930–1965
Liddell(1984)	Not reported	Not reported	ICD 7th	Not reported	1966–1975
Berry(1985)	By examination of histology	Not reported	The Office of Population Censuses and Surveys	Not reported	Men 1933–1955 Women 1936–1942
Reid(2006)	By physician	2000 and 2002	ICD-0 2nd edition categories c33.9-c34.9	Sex, age (±5 years)	1979–2002
Markowitz (2013)	By chest radiographs	1981 and 1983	ICD-9 code 162 (1981–1998) and ICD-10 codes C-33 and C-34 (1999–2006)	Not reported	1982–2008
Wang(2013)	By pathology or biopsy	The first two decades	The Chinese Radiographic Diagnosis Criteria of Pneumoconiosis	Not reported	1981–2006

ICD stands for International Classification of Diseases

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Publication bias: Begg's funnel plot and Egger's test were performed to assess publication bias of the literature. Publication bias for (i) A+S- was $p = 0.437$ (Begg's test), and 0.659 (Egger's), (ii) A-S+ was $p = 0.252$ (Begg's test), and 0.362 (Egger's), and (iii) A+S+, $p = 0.154$ (Begg's test) and 0.294 (Egger's test) suggesting no bias. Funnel plots suggested evidence of publication bias. There was asymmetry of funnel plots accordant with high heterogeneity studies (A-S+ and A+S+). However, trim and fill analysis showed that the overall odds ratios were unchanged (data shown in supplement, S1 Fig).

(ii) Cohort studies: Seven studies [23,24,26,57,63–65] were included in our primary analysis (Fig 3A–3C). The summary relative risks for lung cancer in the cohort studies of (A+S-)

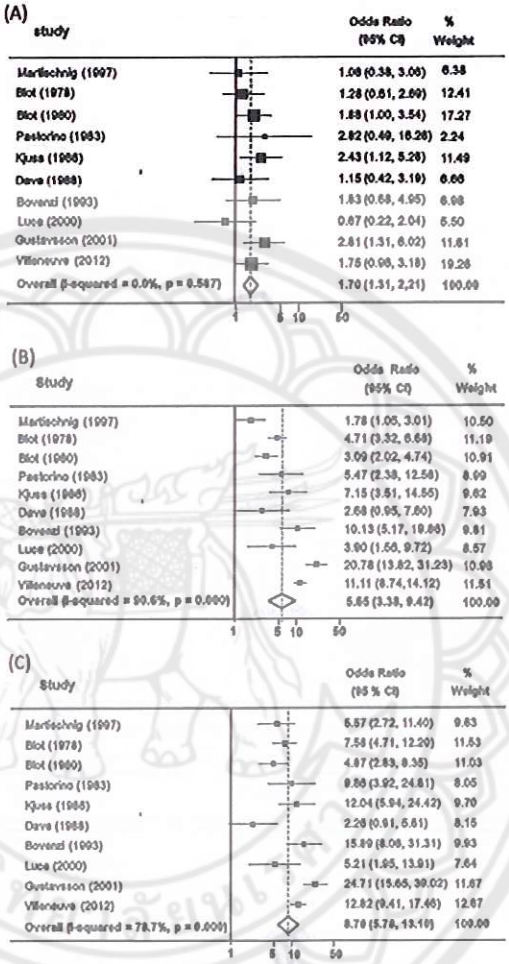

Fig 2. Random-effects meta-analysis of the synergistic effect between asbestos exposure and smoking cause lung cancer- Case control studies. (A) Summary odds ratio of asbestos-exposed and non-smoking (A+S-) compared with not asbestos-exposed and non-smoking (A-S-). (B) Summary odds ratio of non-exposure to asbestos and smoking (A-S+) compared with not asbestos-exposed and non-smoking (A-S-). (C) Summary odds ratio of asbestos-exposed and smoking (A+S+) compared with not asbestos-exposed and non-smoking (A-S-).

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Table 4. Effect of the Exposure to Asbestos (A) and/or Cigarette Smoking (S) on Lung Cancer Risk.

Groups	No. of studies	Reference**	ORs and RRs* (95% CI) A	ORs and RRs* (95% CI) S	ORs and RRs* (95% CI) A and S	*P* for heterogeneity A	*P* for heterogeneity S	*P* for heterogeneity A and S	I^2 (%) A	I^2 (%) S	I^2 (%) A and S
Case-control studies											
Geographic area											
USA	2	1.00	1.60 (0.99–2.59)	3.89 (2.58–5.86)	6.19 (4.01–9.54)	0.435	0.138	0.228	0.0	55.0	31.3
Europe	7	1.00	1.71 (1.15–2.54)	5.63 (2.49–12.71)	8.89 (4.77–16.56)	0.339	0.000	0.000	11.9	90.4	80.3
Study design											
Population Based	6	1.00	1.83 (1.32–2.55)	7.60 (4.09–14.11)	10.92 (6.54–18.22)	0.464	0.000	0.000	0.0	89.7	79.2
Hospital Based	4	1.00	1.49 (0.97–2.29)	3.60 (1.94–6.69)	6.19 (3.47–11.06)	0.501	0.005	0.034	0.0	76.8	65.3
Cohort studies											
Asbestos type											
Chrysotile	3	1.00	2.58 (1.13–5.89)	3.58 (1.75–7.33)	5.04 (2.50–10.18)	0.807	0.798	0.685	0.0	0.0	0.0
Not reported	3	1.00	3.05 (1.53–6.08)	7.33 (4.18–12.85)	10.47 (7.90–13.88)	0.736	0.326	0.501	0.0	10.8	0.0

*Odds ratios is for case-control, relative risk is for cohort study

** Reference is equal one as control group

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workers was 2.72 (95% CI = 1.67–4.40), (A-S+) workers was 6.42 (95% CI = 4.23–9.75), and for (A+S+) workers was 8.90 (95% CI = 6.01–13.18) compared with (A-S-) workers. The results of the cohort studies are consistent with the analysis of the case-control studies. Evidence of heterogeneity was not found in cohort studies (I^2 = 0.0%, p = 0.968, I^2 = 25.1%, p = 0.237 and I^2 = 17.3%, p = 0.298). In addition, case-control studies estimates of the combined effect of asbestos and smoking on lung cancer risk were in concordance with those from cohort studies.

Publication bias: Evaluation of publication bias for A+S-, A-S+ and A+S+ are Begg's test (p = 0.063) Egger's test (p = 0.079), Begg's test (p = 0.026) Egger's test (p = 0.065) and Begg's test (p = 0.118) Egger's test (p = 0.254), respectively. These results did not indicate a potential for publication bias when using funnel plots (data shown in supplement, S2 Fig).

Table 5. Effect of the Exposure to Asbestos (A) and/or Cigarette Smoking (S) on Lung Cancer Risk in Case-Control Studies, Stratified by smoking levels.

Smoking level	No. of studies	ORs (95% CI) A	ORs (95% CI) S	ORs (95% CI) A and S	*P* for heterogeneity A	*P* for heterogeneity S	*P* for heterogeneity A and S	I^2 (%) A	I^2 (%) S	I^2 (%) A and S
Non smokers	2[25,55,56]	2.63 (1.43–4.83)	-	-	0.785	-	-	0.0	-	-
1–19 cigarettes/day	2	-	9.98 (3.44–28.96)	15.38 (7.34–32.24)	-	0.010	0.083	-	85.1	66.8
>20 cigarettes/day	2	-	25.41 (8.96–72.00)	30.31 (15.77–58.25)	-	0.011	0.168	-	84.4	47.5
0–9 cigarettes/day	3[25,55,60]	2.63 (1.57–4.42)	-	-	0.964	-	-	0.0	-	-
10–19 cigarettes/day	3	-	8.54 (2.76–14.76)	13.13 (7.34–32.24)	-	0.000	0.019	-	87.6	74.9
>20 cigarettes/day	3	-	15.76 (4.36–56.94)	25.94 (11.94–56.39)	-	0.000	0.119	-	87.7	53.0

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Interaction between asbestos exposure and cigarette smoking

Evaluation of interaction is summarized in Table 6. All 17 studies provided data which enabled evaluation of the joint effects of co-exposure of both asbestos and cigarette smoking on the risk of lung cancer. For case-control studies, the interaction index of synergy (*S*) and multiplicative index (*V*) were 1.44 (95% CI = 1.26–1.77) and 0.91 (95% CI = 0.63–1.30), respectively, with corresponding values for the cohort studies of 1.11 (95% CI = 1.00–1.28) and 0.51 (95% = 0.31–0.85). These results suggest that the interaction between asbestos exposure and smoking can be a positive interaction on the additive scale (an additive synergistic effect). There was a suggestion of a negative multiplicative interaction for both case-control and cohort studies. Notably our results do not show a multiplicative effect between the two known human carcinogens.

Discussion

Our results demonstrate a positive synergistic interaction on an additive scale between asbestos exposure and cigarette smoking in workers developing lung cancer (Table 6). Employees exposed to asbestos and having a history of smoking have a higher risk of developing lung cancer than those only exposed to one risk (either smoking or asbestos alone). In contrast, the multiplicative index for case-control studies was close to 1.0, although for cohort studies, a negative multiplication interaction is suggested (*V* = 0.51, 95%CI = 0.31–0.85).

Some data suggests that smoking does not enhance mesothelioma [66], which implies that the synergistic lung cancer risk arises from the two carcinogens interacting in the same lung tissue. There are several mediators contributing to cigarette smoke and asbestos-induced lung diseases. Both smoking [67] and asbestos [68] elicit chronic inflammation, which is central to tumorigenesis and is augmented through reduced active immunity, increased infections, and compromised tumor surveillance [69,70]. Tobacco smoke causes inflammation through a vast

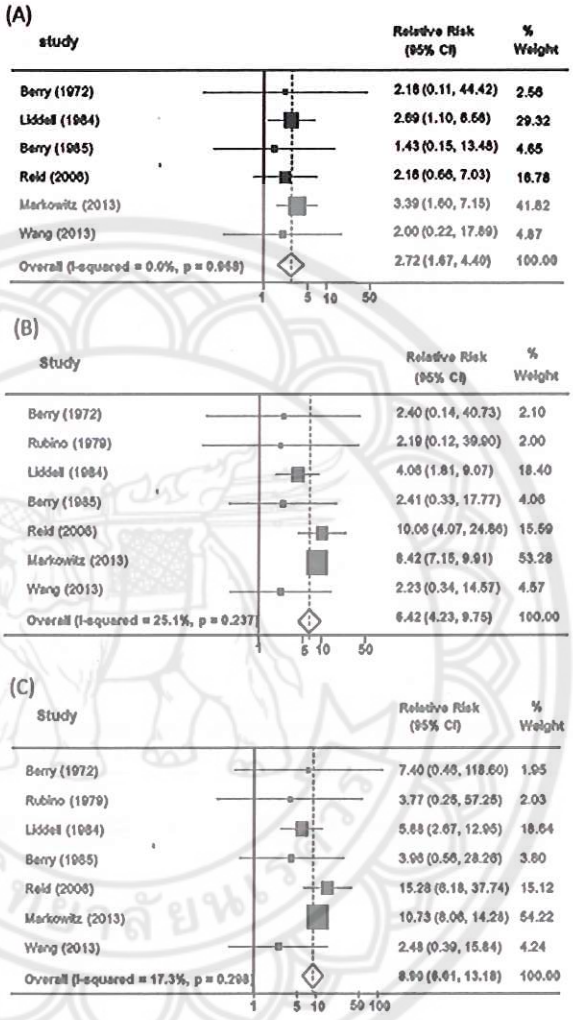

Fig 3. Random-effects meta-analysis of the synergistic effect between asbestos exposure and smoking cause lung cancer- Cohort study. (A) Summary relative risk of asbestos-exposed and non-smoking (A+S-) compared with not asbestos-exposed and non-smoking (A-S-). (B) Summary relative risk of non-exposure to asbestos and smoking (A-S+) compared with not asbestos-exposed and non-smoking (A-S-). (C) Summary relative risk of asbestos-exposed and smoking (A+S+) compared with not asbestos-exposed and non-smoking (A-S-).

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Table 6. Synergy and Multiplicative Indices between Asbestos Exposure and Cigarette Smoking.

Overall risk estimates	Reference	Asbestos	Smoking	Asbestos and smoking	Interaction index* synergy	Interaction index* multiplicative
Odds Ratio	1.00	1.70(1.31–2.21)	5.65(3.38–9.42)	8.70(5.78–13.10)	1.44 (1.26–1.77)	0.91(0.63–1.30)
Relative Risk	1.00	2.72(1.67–4.40)	6.42(4.23–9.75	8.90(6.01–13.18)	1.11 (1.00–1.28)	0.51(0.31–0.85)

* Rothman synergy index

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array of chemical and particulate irritants. Mineral fibers are inflammatory primarily through activation of Nod-like receptor-family protein 3 (NLRP3) of inflammasomes in tissue macrophages. Asbestos fibers evoke vain attacks by macrophages ensuring their continual activation while also adversely affecting function of other immune cells [71,72]. Symptoms of inflammation include oxidative stress, which is worse in blue asbestos (amosite, crocidolite, tremolite) containing Fe ions which generate additional reactive species through Fenton catalysis [73]. The prolonged bio-persistence of these amphiboles further contributes to their greater carcinogenicity than chrysotile and other mineral fibers. Tobacco smoke also contains multiple carcinogens (e.g., 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone or NNK, 1,3-butadiene, ethylene oxide, chromium, polonium-210, arsenic, ethyl carbamate, and hydrazine) that directly interact with DNA [74]. Thus, the common localized inflammatory actions of tobacco smoke and asbestos readily explains additive effects, while the additional actions (direct carcinogenesis and Fenton catalysis) of each insult could account for the additive synergistic interaction.

The present study has some limitations which are mostly inherent in this type of study.

Odds ratios were roughly estimated from the included studies where the measurement methods used and exposure classification varied between studies. For example there were several studies claiming that the duration of asbestos exposure was the same as the period of employment in the workplace. Therefore, short duration jobs reduce the validity and reliability of questionnaires about occupational history. Some studies [58,60,61] did not provide estimates of adjusted risks (age, sex, etc.). The methods used to quantitate exposures to asbestos and cigarette smoke were arbitrary and varied across studies. The type of asbestos used was usually not stated. The diagnosis for lung cancer used different criteria (by physician, chest x-ray, radiography, or information taken from the death certificate). In contrast, other studies have objective exposure and clinical criteria (e.g., Markowitz et al. [24]). The type of lung cancer was rarely stated or even whether mesothelioma was excluded but mesothelioma was never explicitly included. Some case-control studies [55,59] used control populations who had other diseases (e.g., myocardial infarction, bladder cancer, other malignant neoplasms or other lung disease). Most of these diseases are also smoking-related. Nevertheless, all case-control studies endeavored to match controls for confounders. Some studies have data derived from recalling events that took place 10 years or more before the interview/questionnaire, which raises the issue of recall bias and misclassification. Subgroup analysis by smoking level retained high heterogeneity (Table 5) probably due to different methods of data collection and measurement, uncertain duration of smoking (only daily number of cigarettes smoked quoted).

Nevertheless, our study has some strength. It includes new data and the selection criteria complied with the PRISMA and MOOSE guidelines to perform the first systematic review and meta-analysis. Our analysis differed from previous analyses because (i), the strict selection criteria and heterogeneity testing, (ii) testing for statistical interaction (additive and multiplicative). Most studies randomly enrolled greater numbers of control subjects from hospital registers or health authority databases thus reducing selection bias. One study [59] excluded participants who provided incomplete questionnaire data, were non-responders, or who had

emigrated from the area. These unavoidable variations in the study population and diverse methods utilized readily explain the substantial heterogeneity we detected.

While the most dangerous asbestos types are no longer used, other siliceous fibers and chrysotile (in developing nations) are still incorporated into many building products without clear long-term health assessments in humans. Workers exposed to chrysotile showed increased risk of lung cancer (Table 4) [75]. The scientific rigor of cohort studies has improved since the early asbestos work. However, the long latencies for asbestos-induced neoplasms [76] make retrospective study the only practical protocol. Cigarette smoke inhalation and hence airway exposure can be accurately assessed (cigarette numbers, inhalation, filters). However, our study reiterates the difficulty in accurately assessing actual airway exposure to asbestos and was best assessed in the Markowitz et al. study [24]. Personal monitors provided the best indication of exposure but ultimately, only random sputum fiber counts by public health agencies can provide unbiased and accurate measures of exposure. Another problem highlighted by Markowitz et al. [24] and our study is accurately diagnosing the end-stage pathology. Again, monitoring by independent public health authorities is the mechanism most likely to yield accurate reporting. In addition, potential confounders including life-style and especially local air quality data need collecting for the same cohorts.

Conclusion

The present meta-analysis collected and synthesized data currently available and revealed a positive interaction on an additive scale between asbestos exposure and smoking, while showing little evidence of an interaction on a multiplicative scale. The combined effect of asbestos exposure with moderate and heavy smoking in lung cancer suggested a strong positive interaction on an additive scale, i.e., an additive synergism.

Supporting Information

S1 Fig. Funnel plot for 10 case-control studies of relationship between asbestos and cigarette smoking on lung cancer with subjects whom are exposed to asbestos and non-smokers (A), subjects whom are not exposed to asbestos and smokers (B) and subjects whom are exposed to asbestos and smokers (C).
(DOCX)

S2 Fig. Funnel plot for 7 cohort studies of relationship between asbestos and cigarette smoking on lung cancer with subjects whom are exposed to asbestos and non-smokers (A), subjects whom are not exposed to asbestos and smokers (Be) and subjects whom are exposed to asbestos and smokers (C).
(DOCX)

S1 PRISMA Checklist. PRISMA 2009 Checklist.
(DOC)

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Author Contributions

Conceived and designed the experiments: ML YN. Performed the experiments: YN OL WT NC. Analyzed the data: YN OL WT. Contributed reagents/materials/analysis tools: ML OL YN WT CNS BR NC. Wrote the paper: ML OL YN WT CNS BR NC.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA: A Cancer Journal for Clinicians. 2012; 62: 10–29. doi: 10.3322/caac.20138
2. Gandini S, Botteri E, Iodice S, Boniol M, Lowenfels AB, Maisonneuve P, et al. Tobacco smoking and cancer: A meta-analysis. International Journal of Cancer. 2008; 122: 155–164. doi: 10.1002/ijc.23033
3. Lee PN, Forey BA, Coombs KJ. Systematic review with meta-analysis of the epidemiological evidence in the 1900s relating smoking to lung cancer. BMC Cancer. 2012; 12. doi: 10.1186/1471-2407-12-385
4. IARC. Tobacco Smoke and Involuntary Smoking. IARC monographs on the evaluation of carcinogenic risks to humans. 2004; 83: 1–1438.
5. IARC. Tobacco smoking. IARC monographs on the evaluation of carcinogenic risks to humans. 1986; 38: 1–421.
6. (US) Office on Smoking and Health. The Health Consequences of Involuntary Exposure to Tobacco Smoke: A Report of the Surgeon General. Atlanta (GA): Centers for Disease Control and Prevention (US), 2006. Available from: http://www.ncbi.nlm.nih.gov/books/NBK44324/.
7. Hubaux R, Becker-Santos DD, Enfield KS, Lam S, Lam WL, Martinez VD. Arsenic, asbestos and radon: emerging players in lung tumorigenesis. Environmental Health. 2012; 11. doi: 10.1186/1476-069X-11-89
8. Yang M. A current global view of environmental and occupational cancers. Journal of environmental science and health Part C, Environmental carcinogenesis & ecotoxicology reviews. 2011; 29: 223–249. doi: 10.1080/10590501.2011.601848
9. IARC. Asbestos (Chrysotile, Amosite, Crocidolite, Tremolite, Actinolite and Anthophyllite). IARC Monographs on the evaluation of carcinogenic risks to humans. 2012; 100 C.
10. IARC. Asbestos IARC Monographs on the evaluation of the carcinogenic risk of chemicals to humans. 1977; 14: 1–106. PMID: 863456
11. Ferguson DA, Berry G, Jelihovsky T, Andreas SB, Rogers AJ, Fung SC, et al. The Australian Mesothelioma Surveillance Program 1979–1985. Medical Journal of Australia. 1987; 147: 166–172. PMID: 3657627
12. Rake C, Gilham C, Hatch J, Darnton A, Hodgson J, Peto J. Occupational, domestic and environmental mesothelioma risks in the British population: a case-control study. British Journal of Cancer 2009; 100: 1175–1183. doi: 10.1038/sj.bjc.6604879 PMID: 19259084
13. Olsen NJ, Franklin PJ, Reid A, de Klerk NH, Threlfall TJ, Shilkin K, et al. Increasing incidence of malignant mesothelioma after exposure to asbestos during home maintenance and renovation. Medical Journal of Australia. 2011; 195: 271–274. doi: 10.5694/mja11.10125 PMID: 21895596
14. Yano E, Wang X, Wang M, Qiu H, Wang Z. Lung cancer mortality from exposure to chrysotile asbestos and smoking: a case-control study within a cohort in China. Occupational and Environmental Medicine. 2010; 67: 867–871. doi: 10.1136/oem.2009.051615 PMID: 20833758
15. Liddell FD, McDonald AD, McDonald JC. Dust exposure and lung cancer in Quebec chrysotile miners and millers. The Annals of Occupational Hygiene. 1998 42: 7–20. doi: 10.1093/annhyg/42.1.7 PMID: 9566111
16. Nikiński J, Nikińska W, Chyczewska E, Laudański J, Naumnik W, Chyczewski L, et al. The epidemiology of asbestos-related diseases. Lung Cancer 2004; 45: S7–S15. PMID: 15261426
17. Law MR, Ward FG, Hodson ME, Heard BE. Evidence for longer survival of patients with pleural mesothelioma without asbestos exposure. Thorax 1983; 38: 744–746. pmcid: pmc459649. PMID: 6648853
18. Neumann V, Löseke S, Nowak D, Herth FJ, Tannapfel A. Malignant pleural mesothelioma: incidence, etiology, diagnosis, treatment, and occupational health. Deutsches Ärzteblatt International. 2013 110: 319–326. doi: 10.3238/arztebl.2013.0319 PMID: 23720698
19. Nelson HH, Kelsey KT. The molecular epidemiology of asbestos and tobacco in lung cancer. Oncogene 2002; 21: 7284–7288. doi: 10.1038/sj.onc.1205804 PMID: 12379872
20. Vainio H, Boffetta P. Mechanisms of the combined effect of asbestos and smoking in the etiology of lung cancer. Scandinavian Journal of Work, Environment & Health. 1994; 20: 235–242. doi: 10.5271/sjweh.1402

21. Nielsen Elsa, Ostergaard Grete, John Christian Larsen. Toxicological Risk Assessment of Chemicals: A Practical Guide. New York: Informa Healthcare: CRC Press, 2008.

22. Luce D, Bugel I, Goldberg P, Goldberg M, Salomon C, Billon-Galland MA, et al. Environmental exposure to tremolite and respiratory cancer in New Caledonia: a case-control study. American Journal of Epidemiology. 2000; 151: 259–265. PMID: 10670550

23. Wang X, Yano E, Lin S, Yu IT, Lan Y, Tse LA, et al. Cancer mortality in Chinese chrysotile asbestos miners: exposure-response relationships. PLoS One. 2013; 8. doi: 10.1371/journal.pone.0071899

24. Markowitz SB, Levin SM, Miller A, Morabia A. Asbestos, asbestosis, smoking, and lung cancer. New findings from the North American insulator cohort. American Journal of Respiratory and Critical Care Medicine. 2013; 188: 90–96. doi: 10.1164/rccm.201302-0257OC PMID: 23590275

25. Gustavsson P, Nyberg F, Pershagen G, Schéele P, Jakobsson R, Plato N. Low-dose exposure to asbestos and lung cancer: dose-response relations and interaction with smoking in a population-based case-referent study in Stockholm, Sweden. American Journal of Epidemiology. 2002; 155: 1016–1022. doi: 10.1093/aje/155.11.1016 PMID: 12034580

26. Reid A, de Klerk NH, Ambrosini GL, Berry G, Musk AW. The risk of lung cancer with increasing time since ceasing exposure to asbestos and quitting smoking. Occupational and Environmental Medicine. 2006; 63: 509–512. doi: 10.1136/oem.2005.025379 PMID: 16849527

27. Villeneuve PJ, Parent MÉ, Harris SA, Johnson KC, The Canadian Cancer Registries Epidemiology Research Group. Occupational exposure to asbestos and lung cancer in men: evidence from a population-based case-control study in eight Canadian provinces. BMC Cancer. 2012; 12. doi: 10.1186/1471-2407-12-595

28. Sekhon H, Wright J, Churg A. Effects of cigarette smoke and asbestos on airway, vascular and mesothelial cell proliferation. International Journal of Experimental Pathology. 1995; 76: 411–418. pmcid: pmc1997213. PMID: 8652361

29. Jung M, Davis WP, Taatjes DJ, Churg A, Mossman BT. Asbestos and cigarette smoke cause increased DNA strand breaks and necrosis in bronchiolar epithelial cells in vivo. Free Radical Biology and Medicine. 2000; 28: 1295–1299. doi: 10.1016/S0891-5849(00)00211-2 PMID: 10889460

30. Eastman A, Mossman BT, Bresnick E. Influence of asbestos on the uptake of benzo(a)pyrene and DNA alkylation in hamster tracheal epithelial cells. Cancer Research. 1983; 43: 1251–1255. PMID: 6297722

31. Erren TC, Jacobsen M, Piekarski C. Synergy between asbestos and smoking on lung cancer risks. Epidemiology. 1999; 10: 405–411. PMID: 10401875

32. Lee PN. Relation between exposure to asbestos and smoking jointly and the risk of lung cancer. Occup Environ Med. 2001; 58: 145–153. doi: 10.1136/oem.58.3.145 PMID: 11171926

33. Hashim D, Boffetta P. Occupational and environmental exposures and cancers in developing countries. Ann Glob Health. 2014; 80: 393–411. PMID: 25512155

34. Liberati Alessandro, Altman Douglas G, Tetzlaff Jennifer, Mulrow Cynthia, Gøtzsche Peter C, Ioannidis John P A, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. PLOS Medicine. 2009; 6. doi: 10.1136/bmj.b2700

35. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. JAMA. 2000; 283: 2008–2012. doi: 10.1001/jama.283.15.2008

36. Sanderson S, Tatt ID, Higgins JP. Tools for assessing quality and susceptibility to bias in observational studies in epidemiology: a systematic review and annotated bibliography. International Journal of Epidemiology. 2007; 36: 666–676. doi: 10.1093/ije/dym018 PMID: 17470488

37. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. 2013. Available: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

38. Rothman KJ. The estimation of synergy or antagonism. American Journal of Epidemiology. 1976; 103: 506–511. PMID: 1274952

39. DerSimonian R, Laird N. Meta-analysis in clinical trials. Controlled Clinical Trials. 1986; 7: 177–188. doi: 10.1016/0197-2456(86)90046-2 PMID: 3802833

40. Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Statistics in Medicine. 2002; 21: 1539–1558. doi: 10.1002/sim.1186 PMID: 12111919

41. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ. 2003; 327: 557–560. doi: 10.1136/bmj.327.7414.557. pmcid: pmc192859. PMID: 12958120

42. Wong O, Raabe GK. Application of meta-analysis in reviewing occupational cohort studies. Occupational and Environmental Medicine 1996; 53: 793–800. pmcid: pmc1128611. PMID: 8994397

43. Begg CB, Berlin JA. Publication bias and dissemination of clinical research. Journal of the National Cancer Institute. 1989; 81: 107–115. doi: 10.1093/jnci/81.2.107 PMID: 2642556

44. Egger M, Smith GD. Bias in location and selection of studies. BMJ. 1998; 316: 61–66. pmcid: pmc2665334. PMID: 9451274

45. Andersson T, Alfredsson L, Källberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. European Journal of Epidemiology. 2005; 20: 575–579. PMID: 16119429

46. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology. 1992; 3: 452–456. PMID: 1391139

47. Kjuus H, Skjaerven R, Langård S, Lien JT, Aamodt T. A case-referent study of lung cancer, occupational exposures and smoking. I. Comparison of title-based and exposure-based occupational information. Scandinavian Journal of Work, Environment & Health. 1986; 12: 193–202. doi: 10.5271/sjweh.2158

48. Kjuus H, Skjaerven R, Langård S, Lien JT, Aamodt T. A case-referent study of lung cancer, occupational exposures and smoking. II. Role of asbestos exposure. Scandinavian Journal of Work, Environment & Health. 1986; 12: 203–209. doi: 10.5271/sjweh.2157

49. Bovenzi M, Stanta G, Antiga GL, Peruzzo P, Cavalieri F. Occupation and lung cancer risk in the province of Trieste: a case-control study. Medicina del Lavoro. 1992; 83: 338–348. PMID: 1334212

50. de Klerk NH, Musk AW, Armstrong BK, Hobbs MS. Smoking, exposure to crocidolite, and the incidence of lung cancer and asbestosis. British Journal of Industrial Medicine. 1991; 48: 412–417. pmcid: pmc1035388. PMID: 1648376

51. McDonald JC, Liddell FD, Gibbs GW, Eyssen GE, McDonald AD. Dust exposure and mortality in chrysotile mining, 1910–75. British Journal of Industrial Medicine. 1980; 37: 11–24. pmcid: pmc1061328. PMID: 7370189

52. Blot WJ, Davies JE, Brown LM, Nordwall CW, Bulatti E, Ng A, et al. Occupation and the high risk of lung cancer in Northeast Florida. Cancer Research. 1982; 50: 364–371. doi: 10.1002/1097-0142(19820715)50:2<364::AID-CNCR2820500234>3.0.CO;2-Q

53. Morabia A, Markowitz S, Garibaldi K, Wynder EL. Lung cancer and occupation: results of a multicentre case-control study. British Journal of Industrial Medicine. 1992; 49: 721–727. pmcid: pmc1012148. PMID: 1419861

54. Hilt B, Langård S, Lund-Larsen PG, Lien JT. Previous asbestos exposure and smoking habits in the county of Telemark, Norway—a cross-sectional population study. Scandinavian Journal of Work, Environment & Health. 1986; 12: 561–566. doi: 10.5271/sjweh.2106

55. Kjuus H, Langård S, Skjaerven R. A case-referent study of lung cancer, occupational exposures and smoking. III. Etiologic fraction of occupational exposures. Scandinavian Journal of Work, Environment & Health. 1986; 12: 210–215. doi: 10.5271/sjweh.2156

56. Bovenzi M, Stanta G, Antiga G, Peruzzo P, Cavalieri F. Occupational exposure and lung cancer risk in a coastal area of northeastern Italy. International Archives of Occupational and Environmental Health. 1993; 65: 35–41. doi: 10.1007/BF00586056 PMID: 8354573

57. Liddell FD, Thomas DC, Gibbs GW, McDonald JC. Fibre exposure and mortality from pneumoconiosis, respiratory and abdominal malignancies in chrysotile production in Quebec, 1926–75. Annals of the Academy of Medicine, Singapore. 1984; 13: 340–344. PMID: 6497336

58. Martischnig KM, Newell DJ, Barnsley WC, Cowan WK, Fehrmann EL, Oliver E. Unsuspected exposure to asbestos and bronchogenic carcinoma. British Medical Journal. 1977; 1: 748–749. pmcid: pmc1605619. PMID: 856382

59. Blot WJ, Morris LE, Stroube R, Tagnon I, Fraumeni JF Jr. Lung and laryngeal cancers in relation to shipyard employment in coastal Virginia. The Journal of the National Cancer Institute. 1980; 65: 571–575. PMID: 6931936

60. Pastorino U, Berrino F, Gervasio A, Pesenti V, Riboli E, Crosignani P. Proportion of lung cancers due to occupational exposure. International Journal of Cancer. 1984; 33: 231–237. doi: 10.1002/ijc.2910330211

61. Dave S K, Edling C, Jacobsson P, Axelson O. Occupation, smoking, and lung cancer. British Journal of Industrial Medicine. 1988; 45: 790–792. pmcid: pmc1009696. PMID: 3203084

62. Blot WJ, Harrington JM, Toledo A, Hoover R, Heath CW Jr, Fraumeni JF Jr. Lung cancer after employment in shipyards during World War II. New England Journal of Medicine. 1978; 299: 620–624. doi: 10.1056/NEJM197809212991202 PMID: 683235

63. Berry G, Newhouse ML, Antonis P. Combined effect of asbestos and smoking on mortality from lung cancer and mesothelioma in factory workers. British Journal of Industrial Medicine. 1985; 42: 12–18. pmcid: pmc1007410. PMID: 3965010

64. Berry G, Newhouse ML, Turok M. Combined effect of asbestos exposure and smoking on mortality from lung cancer in factory workers. Lancet. 1972; 2: 476–478. PMID: 4115357

65. Rubino GF, Piolatto G, Newhouse ML, Scansetti G, Aresini GA, Murray R. Mortality of chrysotile asbestos workers at the Balangero Mine, Northern Italy. British Journal of Industrial Medicine. 1979; 36: 187–194. pmcid: pmc1008562. PMID: 500777

66. Muscat JE, Wynder EL. Cigarette smoking, asbestos exposure, and malignant mesothelioma. Cancer Research. 1991; 51: 2263–2267. PMID: 2015590

67. Valavanidis Athanasios, Vlachogianni Thomais, Fiotakis Konstantinos, Loridas Spyridon. Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. International Journal of Environmental Research and Public Health. 2013; 10: 3886–3907. doi: 10.3390/ijerph10093886. pmcid: pmc3799517. PMID: 23985773

68. Matsuzaki Hidenori, Maeda Megumi, Lee Suni, Nishimura Yasumitsu, Kumagai-Takei Naoko, Hayashi Hiroaki, et al. Asbestos-induced cellular and molecular alteration of immunocompetent cells and their relationship with chronic inflammation and carcinogenesis. Journal of Biomedicine and Biotechnology. 2012; 2012. doi: 10.1155/2012/492608. pmcid: pmc3304550.

69. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011; 144: 646–674. PMID: 21376230

70. Lee J, Taneja V, Vassallo R. Cigarette smoking and inflammation cellular and molecular mechanisms. Journal of Dental Research. 2012; 91: 142–149. doi: 10.1177/0022034511421200 PMID: 21876032

71. Donaldson K, Murphy FA, Duffin R, Poland CA. Asbestos, carbon nanotubes and the pleural mesothelium: a review of the hypothesis regarding the role of long fibre retention in the parietal pleura, inflammation and mesothelioma. Particle and Fibre Toxicology. 2010; 7. doi: 10.1186/1743-8977-7-5. pmcid: pmc2857820.

72. Nishimura Y, Maeda M, Kumagai-Takei N, Lee S, Matsuzaki H, Weda Y, et al. Altered functions of alveolar macrophages and NK cells involved in asbestos-related diseases. Environmental Health and Preventive Medicine. 2013; 18: 198–204. doi: 10.1007/s12199-013-0333-y. pmcid: pmc3650181. PMID: 23463177

73. Liu G, Cheresh P, Kamp DW. Molecular basis of asbestos-induced lung disease. Annual Review of Pathology: Mechanisms of Disease. 2013; 8: 161–187. doi: 10.1146/annurev-pathol-020712-163942. pmcid: pmc3900296.

74. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. Nature Reviews Cancer. 2003; 3: 733–744. doi: 10.1038/nrc1190 PMID: 14570033

75. Bernstein D, Dunnigan J, Hesterberg T, Brown R, Velasco JA, Barrera R, et al. Health risk of chrysotile revisited. Critical Reviews in Toxicology. 2013; 43: 154–183. doi: 10.3109/10408444.2012.756454 PMID: 23346982

76. Kamp DW. Asbestos-induced lung diseases: an update. Translational Research. 2009; 153: 143–152. PMID: 19304273