

CRYSTAL CHEMISTRY AND PHYSICAL-CHEMICAL CHARACTERIZATION OF MINERAL FIBRES AIMED AT UNDERSTANDING THEIR TOXICITY POTENTIAL

SIMONE POLLASTRI

Dipartimento di Scienze Chimiche e Geologiche, Università di Modena e Reggio Emilia, Via G. Campi 103, 41125 Modena

INTRODUCTION

The general term “mineral fibres” refers to a group of minerals that are ubiquitous on the Earth crust. Among them, the most relevant and, certainly, the most feared ones are asbestos minerals and the fibrous zeolite erionite (Mossman *et al.*, 1990; Baumann *et al.*, 2013), which possess a dreadful reputation because they may provoke fatal lung diseases (mainly lung carcinoma and pleural/peritoneal malignant mesothelioma) through inhalation. Asbestos minerals are further subdivided into two major groups *i.e.*, serpentine asbestos and amphibole asbestos; the fibrous-asbestiform variety of serpentine is called chrysotile and represents the most commonly used form of asbestos. Asbestos fibres are composed of smaller fibrillar components (usually called fibrils; Skinner *et al.*, 1988) clearly visible along a bundle of folded fibres (Fig. 1). This peculiar crystal habit is called fibrous-asbestiform.

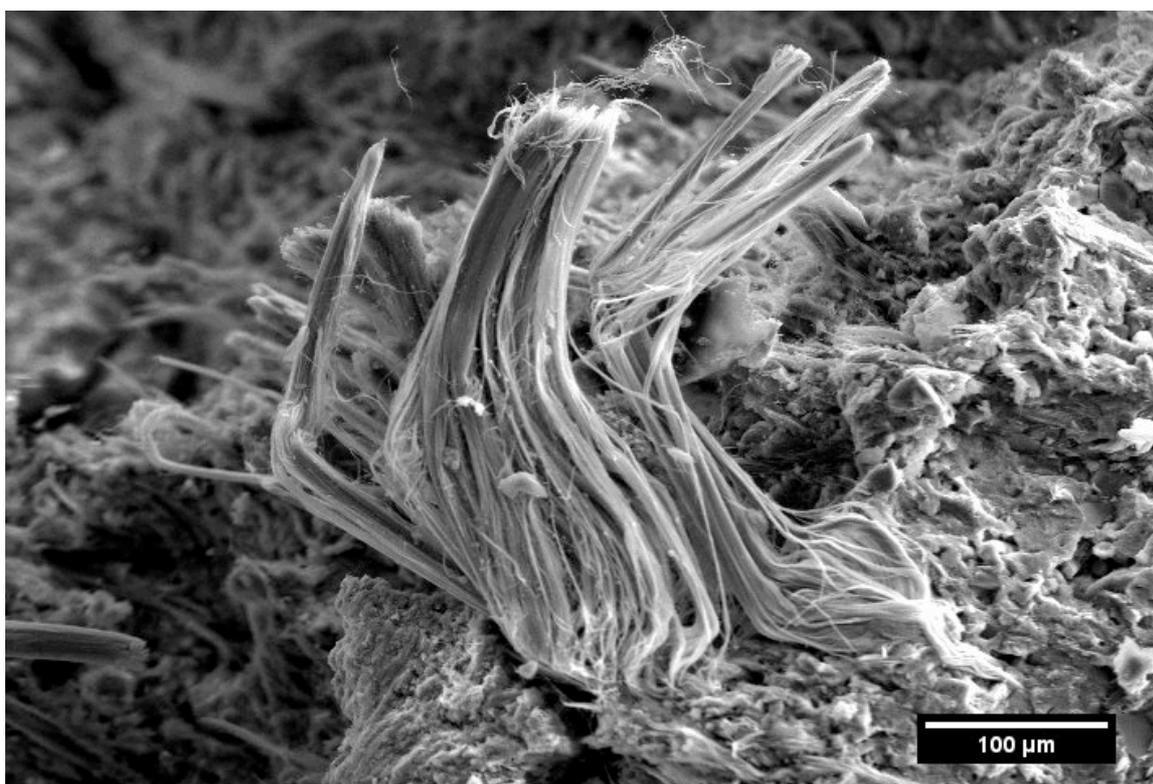


Fig. 1 - SEM image of a bundle of chrysotile fibres.

The family of amphiboles includes five minerals: byssolite (fibrous actinolite), amosite (fibrous grunerite), anthophyllite, crocidolite (fibrous riebeckite) and tremolite. With respect to chrysotile, amphibole fibres are more brittle and exhibit a stiff, needle-like crystal habit. These six asbestos minerals possess outstanding properties that have been exploited in a countless number of mechanical and commercial applications, such as thermal insulation, building materials, fire- and bulletproof- materials, textiles products,

and many others (Gualtieri, 2012). Unfortunately, the fibrous crystal habit, which confers excellent properties to all these minerals, is also the cause of their toxicity potential, due to the release of small inhalable fibres that can reach the lungs and the alveolar sacs (Broaddus, 2001).

The fibrous zeolite erionite represents a special case, both from the chemical-mineralogical and socio-economical point of view. In fact, it does not show an asbestiform crystal habit as that of the asbestos minerals, but is composed of individual fibres of small size. Its fame is primarily linked to the occurrence of erionite-rich sedimentary stones used for building villages in Cappadocia (a region of central Anatolia in Turkey). There, mainly in the three villages of Karain, Tuzcöy and Sarihidir, due to the continuous exposure to erionite fibres, an impressive rate of malignant mesothelioma (MM) causes 50% of deaths in men and women (Bariş *et al.*, 1995; Roushdy-Hammady *et al.*, 2001). Opposite to chrysotile and amphiboles, erionite has never been used for industrial applications.

The “amphibole hypothesis”

In the last two decades, asbestos minerals and erionite have been the subject of intensive multidisciplinary investigations as the mechanisms by which they induce cyto- and genotoxic damage remain poorly understood. In general, the cause-effect relationship between exposure to the fibres and the onset of mesothelioma and other lung diseases remains ambiguous. The difficulties mainly arise from the fact that mineral fibres display great variability in their chemistry, molecular arrangement, size and diameter, surface activity (Pollastra *et al.*, 2014), and ability to generate reactive oxygen species and biopersistence (Donaldson *et al.*, 2010; Pollastra *et al.*, 2014, and references therein) so that drawing a general conclusive model explaining their toxicity has been a pipe dream to date. Because of the existence of this grey area in the scientific knowledge, although it was proven that these mineral fibres, if inhaled, may induce lethal lung diseases (Craighead *et al.*, 1982; Mossman *et al.*, 1996; Becklake *et al.*, 2007; Kamp, 2009) there is still considerable controversy in the scientific community to whether chrysotile asbestos is actually a (potent) carcinogen to humans (Kanarek, 2011; Qi *et al.*, 2013).

In general, the global scientific and political community is divided into two fronts: one side assumes that all above mineral fibres are indistinctly classified as potentially toxic substances; the other side instead promotes the *safe use* of chrysotile assuming that the potential toxicity of this fibre is much lower (or null) with respect to that of fibrous amphiboles and erionite. This latter position relies primarily on the fact that chrysotile is much less biopersistent compared to amphiboles and erionite, and, therefore, it is almost impossible to observe chrysotile fibers in the pleural cavity in the long term (Bernstein, 2014). At the moment, all amphibole asbestos minerals are banned worldwide, whereas chrysotile is banned only in the countries where the line of the International Agency for Research on Cancer (IARC) of the World Health Organization and the National Toxicology Program has been fostered (Mossman & Churg, 1998; Hollan & Smith, 2001; Yano *et al.*, 2001; Roggli *et al.*, 2002; Pfau *et al.*, 2005; Yarborough, 2007). To date, erionite, a human carcinogen listed by the IARC as a Group 1 Carcinogen, surprisingly has not been banned (Dikensoy, 2008).

Aims of the thesis

The purpose of this thesis was the characterization of the major mineral fibres of social and economic-industrial importance (conducted, for the first time, in a systematic way) starting with a full mineralogical-structural and microstructural investigation combined with physical-chemical and biological tests, in order to explain the nature of the biological interaction mechanisms of chrysotile, amphiboles and erionite and draw a conclusive rank of toxicity of mineral fibres.

The final aim was to contribute to the development of a general conclusive model to assess the biological toxicity of mineral fibres. This model would be very useful because there are many mineral fibres (like zeolites) not yet classified that might possess a toxicity potential. By applying the different chemical-physical properties of a not-classified fibre to the model, its potential degree of toxicity could be calculated and new cases of mass exposure, as Biancavilla for fluoro-edenite (Comba *et al.* 2003), and Tuzcöy for erionite (Dumortier *et al.*, 2001) could be avoided.

MATERIALS AND METHODS

Samples selection

The samples investigated in this thesis (Table 1) were eight mineral fibres selected for their socioeconomic and industrial importance.

Table 1 - Nature and details of the investigated mineral fibres.

Sample	Provenance	Notes
Chrysotile	Quebec (Canada) ^a	UICC standard Chrysotile “B” Canadian NB #4173-111-1
Chrysotile	Balangero, Turin (Italy)	
Chrysotile	Val Malenco, Sondrio (Italy)	
Crocidolite	Koegas Mine, Northern Cape (South Africa)	UICC standard Crocidolite South African NB #4173-111-3
Amosite	Penge mine, Northern Province (South Africa)	UICC standard Amosite South African NB #4173-111-4
Fibrous tremolite	Val d’Ala, Turin (Italy)	
Fibrous anthophyllite	Paakkila mine, Paakkila (Finland)	UICC standard Anthophyllite Finnish NB #4173-111-5
Fibrous erionite	Jersey, Nevada (USA)	

^a) Mixture of fibre from the firms Bells, Carey, Cassair, Flintkote, Johns-Manville, Lake, Normandie and National, proportioned roughly to represent Canadian production of asbestos products at that time.

The chrysotile and amphibole samples were selected because they are the mineral fibres that have been most widely used in human history for an endless variety of applications, even if chrysotile is by far the predominant asbestos fibre ever used. Erionite was instead chosen for its high toxicity potential, for its widespread occurrence throughout the world in sedimentary rocks and for its physico-chemical properties, which are considerably different compared to asbestos fibres.

Samples preparation and experimental methods

In order to obtain two distinct size classes of fibres for each sample, gravitational separation in wet conditions were performed using the Appiani levigator method (Jolicoeur *et al.*, 1981). The two obtained size classes were characterized by scanning electron microscope (SEM) analysis in order to verify the outcome of the separation procedure, to check for the chemical composition of the fibres and to estimate the average length of the fibres for each class. Finely powdered samples were also prepared for X-ray Powder Diffraction (XRPD) experiments aimed at structural analysis. Whether this was relatively simple for amphiboles and erionite, it was considerably complicated for the chrysotile samples. For this reason we opted for a cryo-milling process in wet conditions. Simulated Lung Fluids (S.L.F.) solutions were also prepared for the study of the Zeta Potential (indicated by the Greek letter ζ). Specifically, an organic Gamble's modified solution was used (Guldberg *et al.*, 1998). Moreover, in order to explore the characteristics of the fibres after being in contact with cell cultures, representative samples of chrysotile UICC, crocidolite UICC, and erionite were selected and treated with cultured diploid human non-tumorigenic bronchial epithelial (Beas2B) and pleural transformed mesothelial (MeT5A) cells.

In order to fully characterize the selected samples, a combination of several analytical techniques, both using conventional and non-conventional sources, was applied to reach the objectives of the thesis. Specifically, the chemical composition of samples was determined using Electron Microprobe Analysis (EMPA). XRPD and single crystal diffraction experiments were conducted using both conventional and synchrotron radiation sources

(Elettra, Trieste, Italy; SLS, Villigen, Switzerland; ESRF, Grenoble, France), for the determination of impurities and the refinement of the crystal structures. Special attention was given to the surface reactivity of the fibers, through Scanning Electron Microscopy (SEM), X-ray Photoelectron Spectroscopy (XPS), surface area (with the BET method) and ζ potential measurements in different chemical-physical conditions. In addition, the structural environment of iron within the crystalline structures was extensively studied by means of X-ray Absorption (both in the XANES and EXAFS regions) and Fe^{57} Mössbauer spectroscopy. Moreover, samples treated with human cell cultures were characterized using *in situ* μ XANES, μ XRD and μ XRF iron mapping, at the I18 beamline (DLS, Didcot, UK).

RESULTS AND DISCUSSION

Surface reactivity of mineral fibres

The results indicated that chrysotile possesses surface area about 3.3 times higher than amphibole for short fibre samples, and about 4.5 times for long fibres. Erionite evidenced a surface area halfway between chrysotile and amphiboles.

The ζ potential of all samples was investigated to understand the relationship among surface reactivity and fibre pathogenicity.

In double distilled water, chrysotiles showed positive values of ζ potential, whereas crocidolite and erionite showed negative values.

In contact with the Gamble's solution, all fibres displayed negative ζ potential (Fig. 2), clearly showing that this parameter cannot be considered a discriminating factor when it is measured in contact with an organic solution reproducing the cell environment.

In modelling the effects of surface potential of mineral fibres, many physico-chemical parameters, such as hemolytic potential, ROS production, fibre encapsulation, fibre size, and temperature should be considered.

Among these, apoptosis is the major factor within the endoplasmic reticulum stress that activates an unfolded protein response and Ca^{2+} release leading to activation of mitochondria-regulated apoptosis.

Because Ca^{2+} ions are required for the induction of intrinsic apoptosis by mitochondria, the Ca^{2+} ion sequestration by the negatively charged mineral surface may impair the apoptotic response, crucial to counteract the transforming potential of the carcinogenic fibres. Moreover, fibre agglomeration, known to induce the highest biological responses, is favoured by

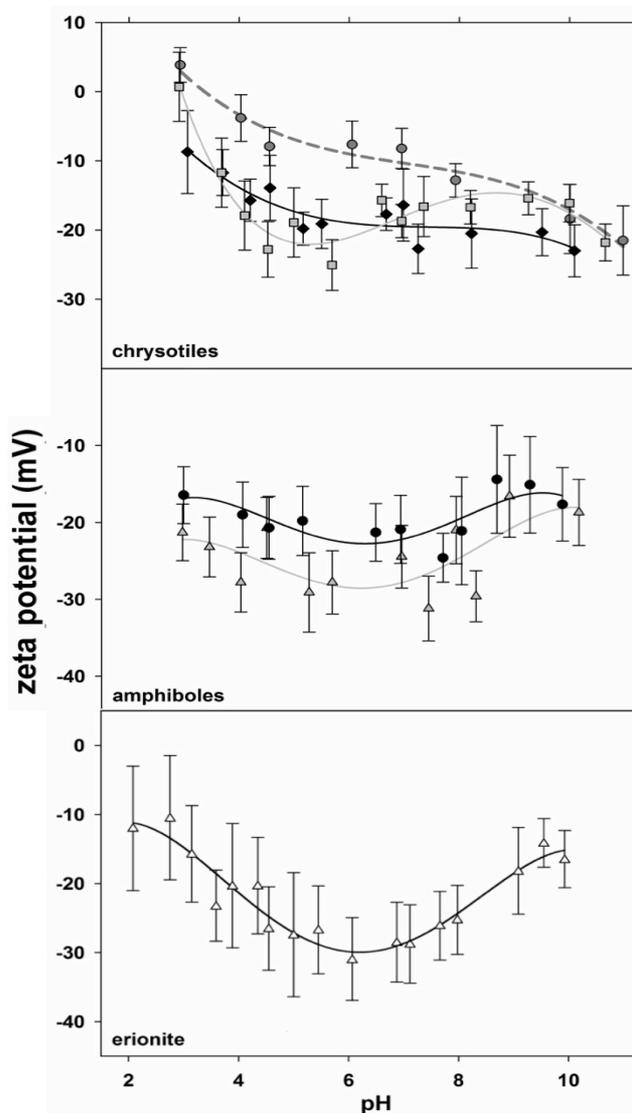


Fig. 2 - Variations of the ζ potential values as a function of pH in Gamble solution at 37 °C on representative samples of chrysotiles, amphiboles and erionite. Legend: (●) chrysotile UICC long; (■) chrysotile Balangero long; (◆) chrysotile UICC short; (●) amosite short; (▲) crocidolite short; (△) erionite short.

the negative values of the ζ potential. All these data are reported in Pollastri *et al.* (2014).

Iron in mineral fibres

From the convergence of XANES, EXAFS and Fe^{57} Mössbauer data, the presence of both Fe^{2+} and Fe^{3+} oxidation states, allocated in octahedral cavities, were detected in all the investigated mineral fibres. This is clearly observed in Fig. 3 (that is a modified version of the variogram from Wilke *et al.*, 2005), where the XANES pre-edge parameters of our samples are plotted against that of standard reference compounds. In amphiboles, Fe^{3+} is in a peripheral octahedral cavity M(2) whereas Fe^{2+} is in an internal octahedral cavity M(1) and M(3). Both Fe^{3+} and Fe^{2+} fill octahedral cavities in chrysotile fibres.

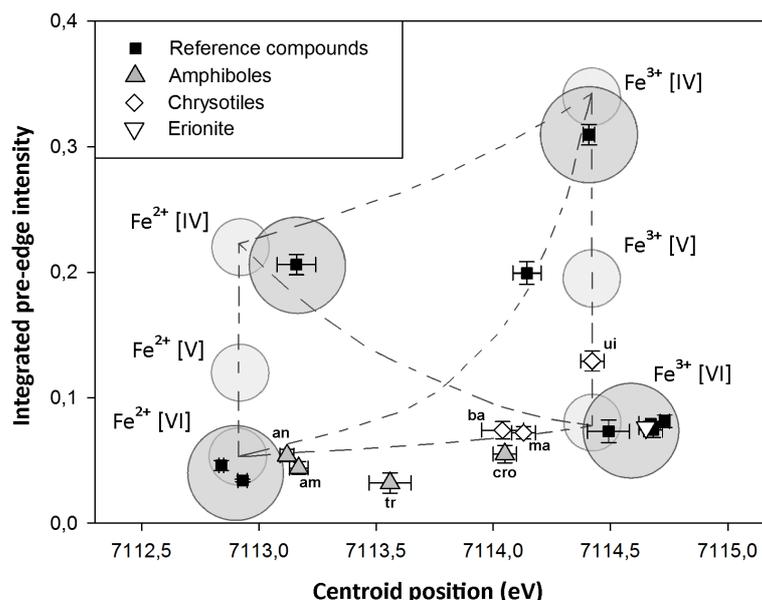


Fig. 3 - Pre-edge parameters of samples and reference compounds plotted in the modified variogram from Wilke *et al.* (2005). Little grey fields designate pre-edge parameters for the Fe co-ordination and oxidation state whereas dashed lines between fields indicate the variation of pre-edge parameters assuming binary mixtures of respective end-members (Wilke *et al.*, 2005); larger grey fields designate our pre-edge parameters.

Taking into account the much shorter dissolution time of chrysotile with respect to amphiboles (although the latter are much more rich in iron) the release of iron could be comparable. This finding may indicate that the overall toxicity potential of chrysotile is not lower than that of amphiboles since production of hydroxyl radicals requires iron to be available at the surface of the mineral fibre in contact with H_2O_2 released in the organic medium, during the persistent inflammatory activity.

Erionite turns out to be a special case since iron seems to be present only in the form of octahedrally coordinated Fe^{3+} particles, although further experimental confirmations are needed.

Considering the dissolution rates and the iron content, a proposed ranking of ability of asbestos fibres to generate “available surface iron-related” (pristine bulk iron made available at the surface of the fibre during the dissolution process) hydroxyl radicals may be: amosite > crocidolite \approx chrysotile > anthophyllite > tremolite. The ranking intentionally does not include erionite, since its toxicity model and the exact location of iron should be validated. The complete data set is reported in Pollastri *et al.* (2015).

Changes of mineral fibres in contact with human cell cultures

Chrysotile UICC, crocidolite UICC, and erionite were exposed to mesothelial MeT5A and broncoalveolar Beas2B cell cultures for 24, 48, 72, and 96 h. Treated samples were investigated using *in situ* synchrotron XRF iron mapping, μ -XANES, and μ -XRD. Results were also supported by a TEM investigation. The contact of the chrysotile fibres with the cell cultures leads to earlier amorphization, which is interpreted as the first dissolution step. Crocidolite shows very minor signs of amorphization whereas erionite seems to be the more stable fibre species in contact with the cells.

The mechanism of amorphization of chrysotile consists of differential dissolution of the Mg-centered octahedral layer: Hargreaves & Taylor (1946) reported that if chrysotile is leached with diluted acid, the magnesia layer can be removed and the structure of original chrysotile becomes amorphous. Seshan (1983) reported that through acid attack, chrysotile surface became silica-like and Mg is lost from the fibres during amorphization. Wypych *et al.* (2005) also reported that the acid-leached product of chrysotile consisted of layered hydrated disordered silica with a distorted structure, resembling the silicate layer existing in the original mineral. Similar observations are also reported in Bernstein *et al.* (2013; Fig. 4).

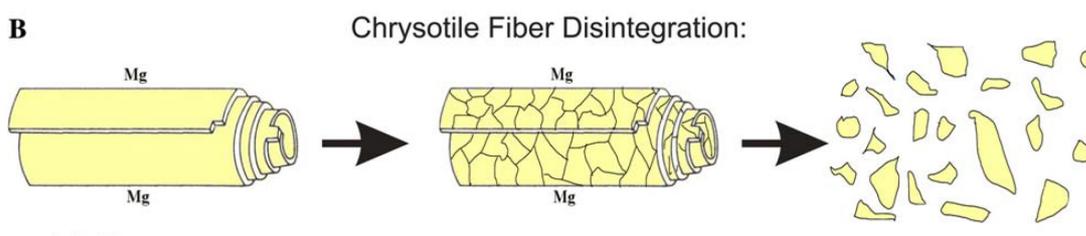


Fig. 4 - Chrysotile Fiber Disintegration: The magnesium is dissolved at neutral pH and the silica matrix is broken up at acid pH (From Bernstein *et al.* 2013).

The formation of a silica-rich fibre skeleton after *pseudo-amorphization* of chrysotile may prompt the production of HO• in synergy with surface iron species; this could indicate that chrysotile may be much more reactive and cytotoxic *in vitro* in the (very) short term whereas the activity of crocidolite and erionite would be much more sluggish but persistent in the long term. Iron in all the fibres at any contact time is represented by Fe³⁺ in octahedral position, with a chemical environment that does not undergo major modifications with respect to the raw samples.

FINAL CONSIDERATIONS

The aim of this thesis was the characterization of the main mineral fibres of social and economic-industrial importance starting with a full mineralogical-structural and microstructural investigations, in order to explain the nature of the biological interaction mechanisms of chrysotile, amphiboles, and erionite and compare them to drawing a convincing rank of toxicity of mineral fibres.

All collected data allowed to state that the mechanisms that lead to the onset of cancer (mainly lung cancer and pleural/peritoneal malignant mesothelioma) are still unclear. But this lack of knowledge is not exclusive of the mineral fibres-related cancers, since although the connection between inflammation and cancer is generally accepted (Grivennikov & Karin, 2010; Gonda *et al.*, 2009), several questions still remain; for example, can inflammation cause neoplasia in the absence of an exogenous carcinogenic agent? (Mantovani *et al.*, 2008). The exact mechanisms by which a wound-healing process turns into cancer are actual topics of intense research (Reuter *et al.*, 2010 and references therein); for these reasons, the only thing that can be said is that in the case the inflammation lasts for a longer period of time compared to acute inflammation (namely chronic inflammation), the host can be predisposed to various chronic diseases, including cancer (Lin & Karin,

2007). From all these considerations, a list of physico-chemical properties that a mineral must possess to be considered potentially toxic, can be defined. It is important to emphasize the fact that it seems to be the simultaneous presence of several factors in determining the toxicity potential of mineral fibers. An excellent example is iron: The presence of active iron present at the surface of the fibres is a key factor of toxicity as it promotes the formation of reactive HO• species by the surface Fenton reaction chain.

Nevertheless, iron-containing particles such as hematite (Craighead & Gibbs, 2008) and magnetite seems to be not active. The explanation could lie in their crystal habit (lamellar for hematite and sub-spherical for magnetite), which promotes full engulfment by macrophage (Champion & Mitragotri, 2006) without ROS production.

So, in order to develop a general model describing the toxicity of mineral fibres, all the physical-chemical characteristics relevant in determining the potential toxicity of a fiber (such as size, presence of iron, biodurability) could be incorporated into a sort of general empirical formula and quantified, in order to gain a final value which is a function of the degree of toxicity potential of that mineral.

This general model of classification (actually in progress) would be very useful to predict *a priori* the toxicity potential of unknown mineral fibre, in order to prevent new cases of mass exposure as that of Biancavilla (Italy) for fluoro-edenite (Comba *et al.*, 2003) and Tuzcöy (Turkey) for erionite (Dumortier *et al.*, 2001).

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REFERENCES

- Bariş, B., Demir, A.U., Shehu, V., Karakoca, Y., Kisacik, G., Bariş, Y.I. (1995): Environmental fibrous zeolite (erionite) exposure and malignant tumors other than mesothelioma. *J. Environ. Pathol. Tox.*, **15**, 183-189.
- Baumann, F., Ambrosi, J.P., Carbone, M. (2013): Asbestos is not just asbestos: an unrecognized health hazard. *Lancet Oncol.*, **14**, 576-578.
- Becklake, M.R., Bagatin, E., Neder, J.A. (2007): Asbestos-related diseases of the lungs and pleura: uses, trends and management over the last century. *Int. J. Tuberc. Lung. Dis.*, **11**, 356-369.
- Bernstein, D.M. (2014): The health risk of chrysotile asbestos. *Curr. Opin. Pulm. Med.*, **20**, 366-370.
- Bernstein, D.M., Dunnigan, J., Hesterberg, T., Brown, R., Velasco, J.A.L., Barrera, R., Hoskins, J., Gibbs, A. (2013): Health risk of chrysotile revisited. *CRC Crit. Rev. Toxicol.*, **43**, 154-183.
- Broaddus, V.C. (2001): Apoptosis and asbestos-induced disease: Is there a connection? *J. Lab. Clin. Med.*, **137**, 314-315.
- Champion, J.A., & Mitragotri, S. (2006): Role of target geometry in phagocytosis. *P. Natl. Acad. Sci. USA*, **103**, 4930-4934.
- Comba, P., Gianfagna, A., Paoletti, L. (2003): Pleural mesothelioma cases in Biancavilla are related to a new fluoro-edenite fibrous amphibole. *Arch. Env. Health*, **58**, 229-232.
- Craighead, J. E., & Gibbs, A.R. (2008): Asbestos and its diseases, Oxford University Press, 403 p.
- Craighead, J.E., Abraham, J.L., Churg, A., Green, F.H., Kleinerman, J., Pratt, P., Seemayer, T.A., Vallyathan, V., Weill, H. (1982): The pathology of asbestos-associated diseases of the lungs and pleural cavities: diagnostic criteria and proposed grading schema. Report of the Pneumoconiosis Committee of the College of American Pathologists and the National Institute for Occupational Safety and Health. *Arc. Pathol. Lab. Med.*, **106**, 544-96.
- Dikensoy, O. (2008): Mesothelioma due to environmental exposure to erionite in Turkey. *Curr. Opin. Pulm. Med.*, **14**, 322-325.
- Donaldson, K., Murphy, F.A., Duffin, R., Poland, C.A. (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. *Part. Fibre Toxicol.*, **7**, 1-17.

- Dumortier, P., Coplü, L., Broucke, I., Emri, S., Selcuk, T., De Maertelaer, V., De Vuist, P., Baris, I. (2001): Erionite bodies and fibres in bronchoalveolar lavage fluid (BALF) of residents from Tuzköy, Cappadocia, Turkey. *Occup. Environ. Med.*, **58**, 261-266.
- Gonda, T.A., Tu, S., Wang, T.C. (2009): Chronic inflammation, the tumor microenvironment and carcinogenesis. *Cell Cycle*, **8**, 2005-2013.
- Grivnenkov, S.I. & Karin, M. (2010): Inflammation and oncogenesis: a vicious connection. *Curr. Opin. Genet. Dev.*, **20**, 65-71.
- Gualtieri, A.F. (2012): Mineral fibre-based building materials and their health hazards. In: "Toxicity of Building Materials", F. Pacheco-Torgal, S. Jalali, A. Fucic, eds. Woodhead, Cambridge, 166-195.
- Guldberg, M., Christensen, V.R., Perander, M., Zoitos, B., Koenig, A.R., Sebastian, K. (1998): Measurement of in-vitro fibre dissolution rate at acidic pH. *Ann. Occup. Hyg.*, **42**, 233-243.
- Hargreaves, A. & Taylor, W.H. (1946): An X-ray examination of decomposition products of chrysotile (asbestos) and serpentine. *Mineral. Mag.*, **27**, 204-216.
- Hollan, J.P. & Smith, D.D. (2001): Asbestos, Clinical Environmental Health and Exposures, 2nd Edition. Lippincott Williams & Wilkins, Philadelphia, 1214-1227.
- Jolicoeur, C., Roberge, P., Fortier, J.L. (1981): Separation of short fibers from bulk chrysotile asbestos fiber materials: analysis and physico-chemical characterization. *Can. J. Chemistry*, **59**, 1140-1148.
- Kamp, D.W. (2009): Asbestos-induced lung diseases: an update. *Transl. Res.*, **153**, 143-152.
- Kanarek, M.S. (2011): Mesothelioma from chrysotile asbestos: update. *Ann. Epidemiol.*, **21**, 688-697.
- Lin, W.W. & Karin, M. (2007): A cytokine-mediated link between innate immunity, inflammation, and cancer. *J. Clin. Invest.*, **117**, 1175-1183.
- Mantovani, A., Allavena, P., Sica, A., Balkwill, F. (2008): Cancer-related inflammation. *Nature*, **454**, 436-444.
- Mossman, B.T. & Churg, A. (1998): Mechanisms in the pathogenesis of asbestosis and silicosis. *Am. J. Resp. Crit. Care*, **157**, 1666-1680.
- Mossman, B.T., Bignon, J., Corn, M., Seaton, A., Gee, J.B. (1990): Asbestos: scientific developments and implications for public policy. *Science*, **247**, 294-301.
- Mossman, B.T., Kamp, D.W., Weitzman, S.A. (1996): Mechanisms of carcinogenesis and clinical features of asbestos-associated cancers. *Cancer. Invest.*, **14**, 466-480.
- Pfau, J.C., Sentissi, J.J., Weller, G., & Putnam, E.A. (2005): Assessment of autoimmune responses associated with asbestos exposure in Libby, Montana, USA. *Environ. Health Persp.*, **113**, 25-30.
- Pollastri, S., Gualtieri, A.F., Gualtieri, M.L., Hanuskova, M., Cavallo, A., Gaudino, G. (2014): The zeta potential of mineral fibres. *J. Hazard. Mater.*, **276**, 469-479.
- Pollastri, S., D'Acapito, F., Trapananti, A., Colantoni, I., Andreozzi, G.B., Gualtieri, A.F. (2015): The chemical environment of iron in mineral fibres. A combined X-ray absorption and Mössbauer spectroscopic study. *J. Hazard. Mater.*, **298**, 282-293.
- Qi, F., Okimoto, G., Jube, S., Napolitano, A., Pass, H.I., Laczko, R., Demay, R.M., Khan, G., Tiirikainen, M., Rinaudo, C., Croce, A., Yang, H., Gaudino, G., Carbone, M. (2013): Continuous exposure to chrysotile asbestos can cause transformation of human mesothelial cells via HMGB1 and TNF- α signaling. *Am. J. Pathol.*, **183**, 1654-1666.
- Reuter, S., Gupta, S.C., Chaturvedi, M.M., Aggarwal, B.B. (2010): Oxidative stress, inflammation, and cancer: how are they linked? *Free Radical Bio. Med.*, **49**, 1603-1616.
- Rogli, V.L., Vollmer, R.T., Butnor, K. J., Sporn, T.A. (2002): Tremolite and mesothelioma. *Ann. Occup. Hyg.*, **46**, 447-453.
- Roushdy-Hammady, I., Siegel, J., Emri, S., Testa, J.R., Carbone, M. (2001): Genetic-susceptibility factor and malignant mesothelioma in the Cappadocian region of Turkey. *Lancet*, **357**, 444-445.
- Seshan, K. (1983). How are the physical and chemical properties of chrysotile asbestos altered by a 10-year residence in water and up to 5 days in simulated stomach acid? *Environ. Health Persp.*, **53**, 143-148.
- Skinner, H.C.W., Ross, M., Frondel, C. (1988) Asbestos and other fibrous materials. Mineralogy, crystal chemistry, and health effects. Oxford University Press, New York, 204 p.
- Wilke, M., Partzsch, G.M., Bernhardt, R., Lattard, D. (2005): Determination of the iron oxidation state in basaltic glasses using XANES at the K-edge. *Chem. Geol.*, **220**, 143-161.
- Wypych, F., Adad, L.B., Mattoso, N., Marangon, A.A.S., Schreiner, W.H. (2005): Synthesis and characterization of disordered layered silica obtained by selective leaching of octahedral sheets from chrysotile and phlogopite structures. *J. Colloid Interf. Sci.*, **283**, 107-112.
- Yano, E., Wang, Z.M., Wang, X.R., Wang, M.Z., Lan, Y.J. (2001): Cancer mortality among workers exposed to amphibole-free chrysotile asbestos. *Am. J. Epidemiol.*, **154**, 538-543.
- Yarborough, C.M. (2007): The risk of mesothelioma from exposure to chrysotile asbestos. *Curr. Opin. Pulm. Med.*, **13**, 334-338.