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Review

Journey to the centre of the lung. The perspective of a mineralogist on the carcinogenic effects of mineral fibres in the lungs

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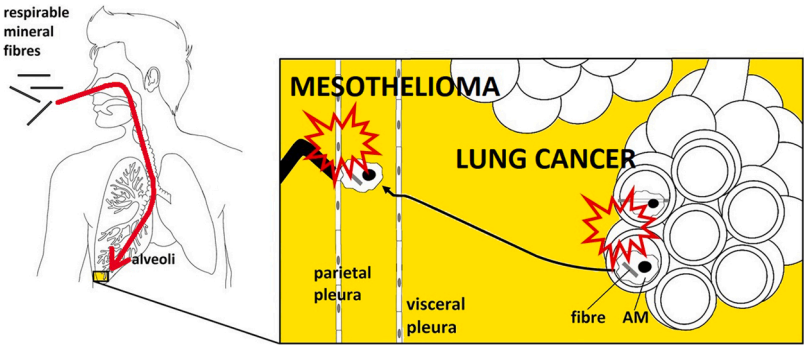
HIGHLIGHTS

- Adverse effects produced in the lungs by mineral fibres are reviewed.
- The carcinogenic fibres crocidolite, chrysotile and erionite are selected as case studies.
- The behaviour of the fibres in vivo is correlated to their crystal-chemistry and properties.
- Attention is given to the cause-effect relationship for lung cancer and malignant mesothelioma.
- Open issues, data gaps, and conflicts in the literature are highlighted.

GRAPHICAL ABSTRACT



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ABSTRACT

This work reviews the bio-chemical mechanisms leading to adverse effects produced when mineral fibres are inhaled and transported in the lungs from the perspective of a mineralogist. The behaviour of three known carcinogenic mineral fibres (crocidolite, chrysotile, and fibrous-asbestiform erionite) during their journey through the upper respiratory tract, the deep respiratory tract and the pleural cavity is discussed. These three fibres have been selected as they are the most socially and economically relevant mineral fibres representative of the classes of chain silicates (amphiboles), layer silicates (serpentine), and framework silicates (zeolites), respectively. Comparison of the behaviour of these fibres is made according to their specific crystal-chemical assemblages and properties. Known biological and subsequent pathologic effects which lead and contribute to carcinogenesis are critically reviewed under the mineralogical perspective and in relation to recent progress in this multidisciplinary field of research. Special attention is given to the understanding of the cause-effect relationships for lung cancer and malignant mesothelioma. Comparison with interstitial pulmonary fibrosis, or "asbestosis", will also be made here. This overview highlights open issues, data gaps, and conflicts in the literature for these topics, especially as regards relative potencies of the three mineral fibres under consideration for lung cancer and mesothelioma. Finally, an attempt is made to identify future research lines suitable for a general comprehensive model of the carcinogenicity of mineral fibres.

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1. Introduction

More than 400 mineral species having a fibrous crystal habit occur in nature (Gualtieri, 2017). Among them, "asbestos" species and fibrous/asbestiform erionite are considered the most hazardous (Wylie, 2017). "Asbestos" minerals are taken generally to include chrysotile (a member of the serpentine group) and five asbestiform amphibole species: actinolite asbestos, amosite, anthophyllite asbestos, crocidolite and tremolite asbestos (IARC, 2012). Erionite (from this point "erionite" will be used to refer to "fibrous/asbestiform erionite) is a naturally occurring fibrous zeolite (Gottardi and Galli, 2012). All six "asbestos" species and erionite are considered by the International Agency for Research on Cancer (IARC) as *"carcinogenic to humans (Group 1)"* (IARC, 2012). In this review, mesothelioma and cancer of the lung will be considered and integrated with some comments on interstitial pulmonary fibrosis or "asbestosis" (American Thoracic Society, 1986).

It is generally accepted that the physical-chemical characteristics of mineral fibres and morphometric parameters (e.g., length, width, aspect ratio) play a key role in carcinogenesis (Wylie et al., 1993, 2022; Borm et al., 2004; Bernstein, 2022). According to a generic model we refer to here as the "fibre toxicity paradigm", long, thin, and biodurable "asbestos" fibres may reach the alveolar and pleural/peritoneal spaces where they induce adverse effects and chronic inflammation. Suggestions have been made whereby the latter effects may ultimately be involved in the onset and propagation (initiation and promotion) of lung malignancies (Stanton et al., 1981; Donaldson et al., 2010; Carbone et al., 2019). Carcinogenesis for these fibres is described here by complex multistep mechanisms governed by the interplay of structural and physicochemical characteristics, including morphology, chemical composition, surface activity, and biodurability/biopersistence (Bernstein et al., 2005, 2021).

The biochemical mechanisms leading to adverse effects in vivo caused by the inhalation of crocidolite, chrysotile, or erionite, as representative of the classes of chain silicates (amphiboles) that include all hazardous commercial amphibole species (namely amosite), layer silicates (serpentine), and framework silicates (zeolites), respectively, are reviewed.

Here, the perspective of the mineralogist is used for comparing the behaviour of the different mineral fibres within the lungs with respect to their chemistry and crystallinity and physical-chemical properties. Our understanding of fibre activity in vivo is constructed using data from the mineral fibre literature. Differences in behaviour of these fibres within the upper respiratory tract, the deep respiratory tract, and the pleural cavity are discussed with respect to their different crystal-chemical structures. The previously published literature, traditional perspectives, and recent progress in mineralogical studies are critically reviewed in an attempt to construct a general unified picture for understanding how the physical-chemical parameters of fibres contribute to the initiation and promotion of lung cancer and malignant mesothelioma (MM). This description relies on both consolidation of previous evidence and coherent interpretation of that evidence, taking into account newer information from experimentation. It highlights debated open issues, especially as to relative carcinogenic potency for the different fibre types in causation of malignancy in lung and pleura.

The comparison of the three different types of mineral fibres is concerned with the fate of each relative to that of the others. Although a comprehensive picture is attempted, this overview does not claim to be exhaustive with regard to biological or medical aspects. For example, this review does not consider exposure and dose rates (Barrett et al., 1989; Mossman et al., 1990), which are known to play a key role in determining overall carcinogenic potential. It is also known that, although precise threshold limit values have still not been defined, dose-response experiments suggest that there is a threshold for pathologic responses, as is well demonstrated for chrysotile exposures in human lung cancers (Pierce et al., 2016). We recognize suggestions of a role for other influencing factors such as "asbestos"-related release of polycyclic aromatic hydrocarbons (PAH) and other chemicals (Kennedy and Little, 1974; Mossman and Craighead, 1981, 1982) and smoke (Keeling et al., 1993) but do not try to incorporate these in this review. Translocation to other organs with corresponding adverse effects are also not considered here.

A glossary of the terms used in this manuscript is provided in Appendix I (Supplementary material).

2. Fibres in the upper respiratory tract

Occupational or environmental exposures may lead to inhalation of (mineral) fibres. Fibres that are capable of passing through the upper respiratory system and reaching the deep respiratory system are "respirable fibres", defined by WHO as "any elongated inorganic particle having length $\geq$ 5 μm, width $\leq$ 3 μm, and length/diameter ratio $\geq$ 3:1" (WHO, 2000). Aerodynamics of this movement are governed mostly by fibre diameter (Gualtieri et al., 2017).

Fibres like any other inhaled airborne particles can be blocked directly by the nasal cilia. The body's next defence mechanism against penetration of foreign bodies, including fibres, is mucociliary clearance via activation of the "mucociliary escalator" (Oberdörster, 1993). This mechanism helps to keep deep lungs free of inhaled material even when exposed to a heavily polluted atmosphere, up to the point of overload (Clarke and Demetri, 2015). The path of the fibres through the respiratory system are shown in Fig. 1.

The bronchi are lined with ciliated columnar cells and the cilia behave as "beaters", transporting particles on a layer of mucus overlying their tips upward to the larynx where they can be expelled by coughing or by swallowing (Skinner et al., 1988). Ciliary clearance of mucus from the lungs can be seen as an escalator with increasing speed the closer it gets to the mouth (Clarke and Demetri, 2015).

Based on consolidated models (Yeh et al., 1976; Heyder et al., 1986), very large particles with $D_{ae} > 5$ μm are deposited in the nasal respiratory tract, particles with $D_{ae} \geq 3$ μm are deposited in the laryngeal and bronchial respiratory tract, and particles with $D_{ae} < 3$ μm are subject to maximum alveolar particle deposition.

Given that many physico-chemical parameters influence this deposition of inhaled materials along the respiratory tract (Oberdörster, 1993), particle deposition is determined by three mechanisms as described by Borm and Donaldson (2007):

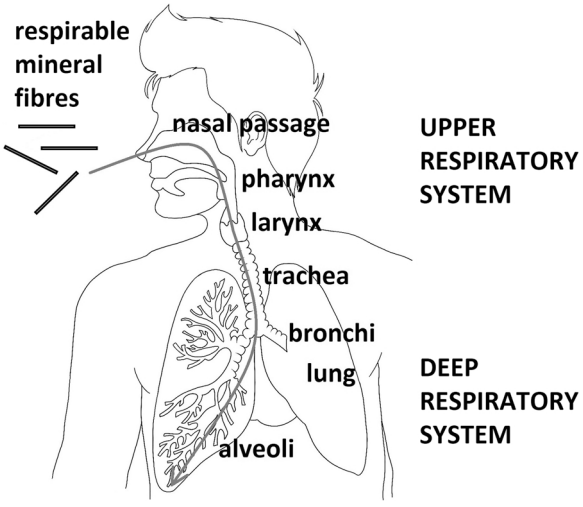

Fig. 1. A sketch of the path that respirable fibres do through the nose, in the upper respiratory system, all the way down to the alveolar space in the deep respiratory system.
(modified after an original picture by Gualtieri A.R.)

(1) *impaction* along airway walls by their inertial mass in branching airways, acting on larger particles with aerodynamic diameter $D_{ae} > 1.5$ μm;
(2) *sedimentation* by gravitational forces acting on particles with $D_{ae} > 0.5$ μm and < 1.5 μm;
(3) *diffusion* of the smallest particles with $D_{ae} < 0.5$ μm through thermal motion of air molecules.

3. Fibres in the deep respiratory tract

As noted above, fibres having $D_{ae} < 3$ μm have the maximum probability for reaching the alveoli. If they reach this compartment, they are in direct contact with the lung lining fluid consisting of an alveolar subphase fluid and pulmonary surfactant (70–500 nm thick layer) secreted by type II alveolar epithelial cells. The latter prevents collapse of the alveolar space during breathing, through relief of surface tension (Creuwels et al., 1997). Fluids in this alveolar gas exchange region contain a higher concentration of proteins than the upper respiratory tract (Hatch, 1992; Innes et al., 2021). Albumin is the most abundant protein (up to 50 % of the total protein content in the lung extracellular fluid) with subordinate immunoglobulins, transferrin, lysozyme, the proteases cathepsin D, dipeptidyl peptidase 4, elastase, ascorbate and others (Innes et al., 2021).

Surfactant secreted by alveolar epithelial cells is composed of approximately 10 % surfactant, proteins (SP-A, SP-B, SP-C and SP-D) and 90 % lipids. The latter are mostly phospholipids like dipamitoylphosphatidylcholine (DPPC) (41–70 %) and minor neutral lipids such as cholesterol, triglycerides and free fatty acids (Lang et al., 2005; Innes et al., 2021) that offer the aforementioned protection against alveolar collapse, as well as providing further support in defence of the lungs.

Fibres reaching the alveolar space are subject to two biochemical actions:

(i) detoxification within lung lining fluid by metal binding proteins, specifically glycoproteins such as albumin (see above) and mucin (Bal et al., 2013; Innes et al., 2021);
(ii) the clearance action of alveolar macrophages (AM) (described in the next section).

Detoxification occurs when the metal binding proteins form a protein corona on the particle surface, leading to surface modifications. These may cause significant differences in particle chemical behaviour in vivo (Monopoli et al., 2011). In this regard, Vigliaturo et al. (2022) recently confirmed at a nanoscale level the presence of an altered surface layer of amphibole fibres in contact with alveolar epithelial cells. This phenomenon has proven to be significant for nanoparticles (NP) that can be fully covered and engulfed within a layer of proteins of different nature. Raesch et al. (2015) characterised this protein corona formed in pulmonary surfactant after NP inhalation and observed remarkably variable assemblages consisting of more than 400 different proteins.

3.1. Clearance of the fibres by alveolar phagocytes

The second major biological response to fibres' arrival into alveolar spaces is the clearance action of alveolar macrophages (AM). AM, with diameter ≤ 21 μm (Krombach et al., 1997; Skinner et al., 1988; Hussell and Bell, 2014) and average lifetime of 60 days, are a population of cells generally differentiated from bone marrow precursors (myeloid cells) that belong to the innate immune system. They have a fundamental role in both triggering and maintaining the inflammatory response which develops in tissues when presented with foreign bodies (Skinner et al., 1988) and accumulate in tissue in response to homeostatic chemokines released from injured cells (Gordon, 2007). Once recruited at the site of inflammation in the specific tissue, macrophages have the capacity to adapt their phenotype in response to different local *stimuli*, displaying a high degree of morphologic plasticity. In the lungs, AM are rapidly recruited at the site of injury and initiate the inflammatory process. Inflammation in turn prompts the release of homeostatic chemokines (Borish and Steinke, 2003; Davenport, 2009; Zlotnik et al., 2011), further engaging circulating monocytes, causing their extravasation and maturation in tissue to non-activated (M0) macrophages. Subsequently, these newly differentiated M0 macrophages are polarized by local *stimuli* in the tissue, to phenotypes defined as either classically activated type 1 (M1), or alternatively activated type (M2) macrophages (Arora et al., 2018). Activated M1 macrophages (but also lung epithelial cells) release pro-inflammatory cytokines (like TNF-α, IL-1β, IL-6, IL-12, IL-15), and promote the production of reactive oxygen species (ROS), proteolytic enzymes and bioactive lipids. TNF-α, in turn, stimulates the release of α-chemokines (IL-8 and IP-10 for example) or β-chemokines (like MCP-1, MIP and RANTES) mediators of inflammation via paracrine and autocrine pathways (Driscoll et al., 1997; Comar et al., 2016). M2 macrophages, which can be polarized by IL-4 and/or IL-13 stimuli (Rhee, 2016), may appear later at the site of inflammation and counteract the pro-inflammatory activity of the M1 phenotype by secretion of anti-inflammatory mediators (e.g., IL-10, CCL18, CCL22) and tissue repair promoting factors, such as TGF-β, VEGF and FGF (Martinez et al., 2009). It is now recognized that macrophages exist as several subpopulations having different levels of M1 or M2 markers and varied activities (Laskin et al., 2019).

In the process called phagocytosis, there is an absorptive clearance mechanism, in which dissolution is the main mechanism, and a non-absorptive clearance mechanism without dissolution (Keller et al., 2020). The two possibly act in synergy:

(a) A biochemical-physical (absorptive) clearance during which the macrophage engulfs the fibre, generates an organelle phagosome sac that surrounds it, and moves the phagosome-particle cluster toward the centrosome of the phagocyte. There, the complex is fused with a lysosome, forming a phagolysosome with an acidic environment that leads to the degradation of the engulfed particle. Lysosomes contain hydrolytic enzymes (proteases, peptidases, nucleases, phosphatases, and glycosidases) while lipids and proteins compose the lysosome membrane. The lysosome environment is complex and undergoes changes with internalisation of exogenous material and is also rich in chelating agents, including citrate and bicarbonate (Tappel, 1969). The acidic environment in the phago-lysosome reflects the dynamic endocytosis process: the pH is expected to range from 6.0 to 6.5 in early endosomes, 5.0–6.0 in late endosomes, and 4.5–5.0 in lysosomes (Schmaljohann, 2006). In fact, the scale of lysosome pH may be even greater; for example, optimum hydrolysis of some structural amino-acids components, or of peptides, can lead to pH 7.2 and 7.8, respectively, while optimal hydrolysis of albumin can occur at pH as low as 2.5 (Tappel, 1969). Low pH is known to contribute to the dissolution of mineral fibres (Rozalen et al., 2013) with a reaction starting at the surface of the fibre and mediated by several factors, including chelating agents (citrate, oxalate, tartrate, ethylenediaminetetraacetic acid, glycine, lactate) responsible for mechanisms such as complexation, redox activity, proton-promoted dissolution, or enzymatic action (Innes et al., 2021). Even though this low acidity "attack" may not completely dissolve the fibre, it can break it into pieces, allowing for more efficient fibre clearance (Eastes et al., 2007). Without such breakage, it is difficult to explain how fibres in general could clear so rapidly from the lungs (Eastes and Hadley, 1995). Residues of the lysosome fibre dissolution are eventually processed down to single ions or molecules (for example, hydrated Mg atoms and metals, hydrated silicic acid for chrysotile from chrysotile) and expelled in situ (exocytosis). Alternatively, macrophages can transport these residues back to the upper respiratory system, followed by mucociliary propulsion towards the oropharynx (Keller et al., 2020). Macrophages may also

translocate from the lung parenchyma to the ~ 20 μm thick pleural cavity, to release their residue cargo through *diaphragmatic stomata*, the 3–10 μm wide (Donaldson et al., 2010) openings at the parietal pleura serving as gates to the lymphatic system (hilar, mediastinal and parasternal lymph nodes and posterior mediastinal lymphoid tissue) (case **(a)** in Fig. 2).

(b) A physical (non-absorptive) clearance during which the macrophage carries or engulfs (but does not dissolve) the fibre. The macrophage-fibre aggregate can move back to the upper respiratory system where mucociliary propulsion towards the oropharynx occurs (Keller et al., 2020) or can migrate to the pleural cavity expelling fibre through stomata in the lymphatic system (case (**b**) in Fig. 2).

Successful clearance via phagocytosis is possible only if the fibre length is < 10 μm, because macrophages can effectively engulf particles of that size or smaller (Donaldson et al., 2010). Stanton (1973) first postulated that "asbestos" pathogenicity is related to biodurable fibres with length > 10 μm. In particular, fibres with length > 20 μm are cleared more slowly from the respiratory system because they cannot be engulfed by macrophages, leading to "frustrated phagocytosis" (Searl et al., 1999). Recent contributions also confirm that size is a factor of paramount importance in determining the carcinogenicity potential of "asbestos" fibres (Wylie et al., 2022) with shorter fibres (e.g. with length <5 μm) that are assumed to have little if any carcinogenic effect (Bernstein, 2022).

3.2. Translocation of the fibres in the pleural cavity

The model of successful phagocytosis is simple and well described except for a mechanism of translocation from the alveolar spaces to the pleural cavity. While there seems to be no doubt that fibres do translocate to the pleural cavity (a process called "primary translocation"), the overall mechanism is still obscure (Coin et al., 1992; Cugell and Kamp, 2004). This "pleural drift" was first experimentally observed in rats by Choe et al. (1997).

Before the possible pathways for fibres' translocation are discussed, it is important to point out that doubts may arise on the actual necessity for fibres to translocate to the pleura to cause an adverse effect. We know for example that amphiboles tend to be deposited not only in the deep lung, but to be deposited in the periphery of the deep lung. We also know from animal studies that (at least in the peritoneum), cytokines, chemokine and ROS production can be generated by the presence of fibres without any physical contact of those fibres with the target cells. Why could this not be true in the pleura as well? It seems at least intuitive that if inflammation is what leads to malignancy via the production of chemokines, cytokines, and reactive species, it is not necessary that the fibres themselves actually enter the pleural cavity, only that the responsible substances can actually cross the pleural membrane and affect the target cells.

Said that, possible pathways for either free or macrophage-engulfed fibres (Miserocchi, 1997; Miserocchi et al., 2008; Donaldson et al., 2010) include:

(a) Paracellular transit (through the space between the cells) from the parenchyma to the pleural cavity (path (**a**) in Fig. 3**a**), down the gradient for physiological water adsorption. This process can be repeated over and over until the fibres eventually reach the pleural cavity by piercing the visceral mesothelial cells. The driving force of the particle's movement is the negative pressure (−6 to −8 cm H_2O at inspiration and−2 to −4 cm H_2O at expiration) in the pleural cavity (Toyokuni, 2019);

(b) A mechanism similar to (a) but expedited by fenestration in the epithelial lining (path (**b**) in Fig. 3a). In this mechanism, macrophages induce inflammation leading to the death of alveolar epithelial cells. Subsequent desquamation of the alveolar epithelial lining opens a preferred paracellular path to the pleural cavity. Both the normal flow of lymph and the normal trans-

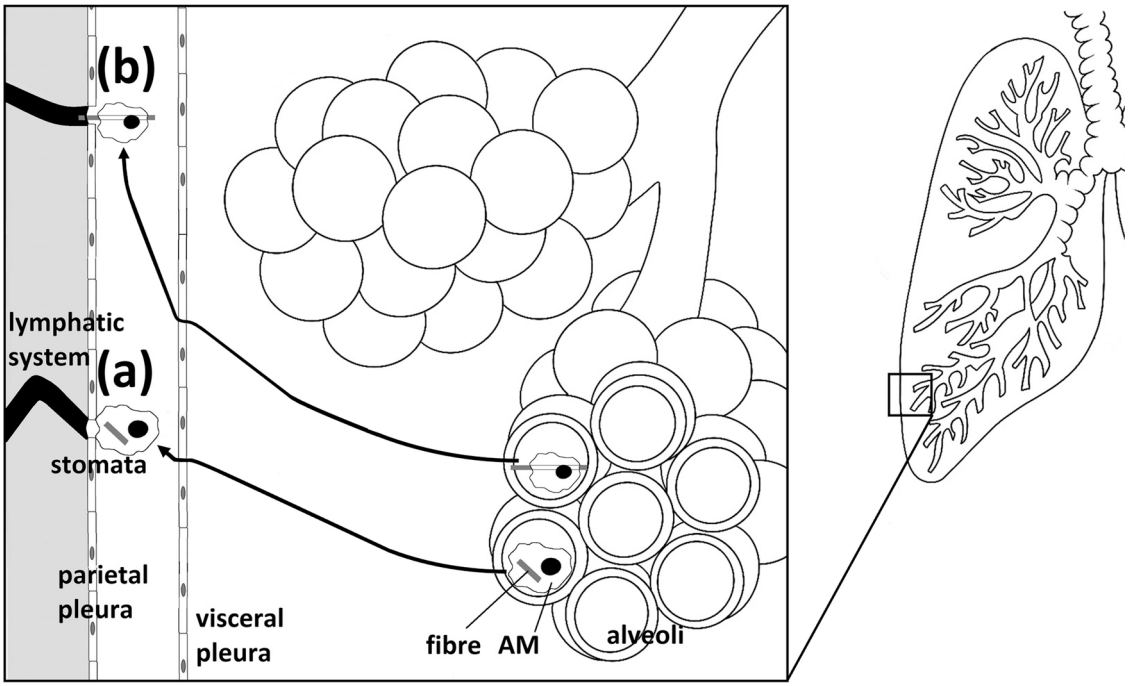

Fig. 2. path (**a**) translocation of the alveolar macrophage (**AM**) with engulfed short fibre through the parenchyma and the visceral pleura into the pleural space to approach the stomata (openings at the parietal pleura) and release the engulfed material in the lymphatic system; path (**b**) translocation of the alveolar macrophage (**AM**) with carried or partially engulfed long fibre through the parenchyma and the visceral pleura into the pleural space to approach the stomata (openings at the parietal pleura) in the attempt to release the fibre in the lymphatic system.
(modified after an original picture by Gualtieri A.R.)

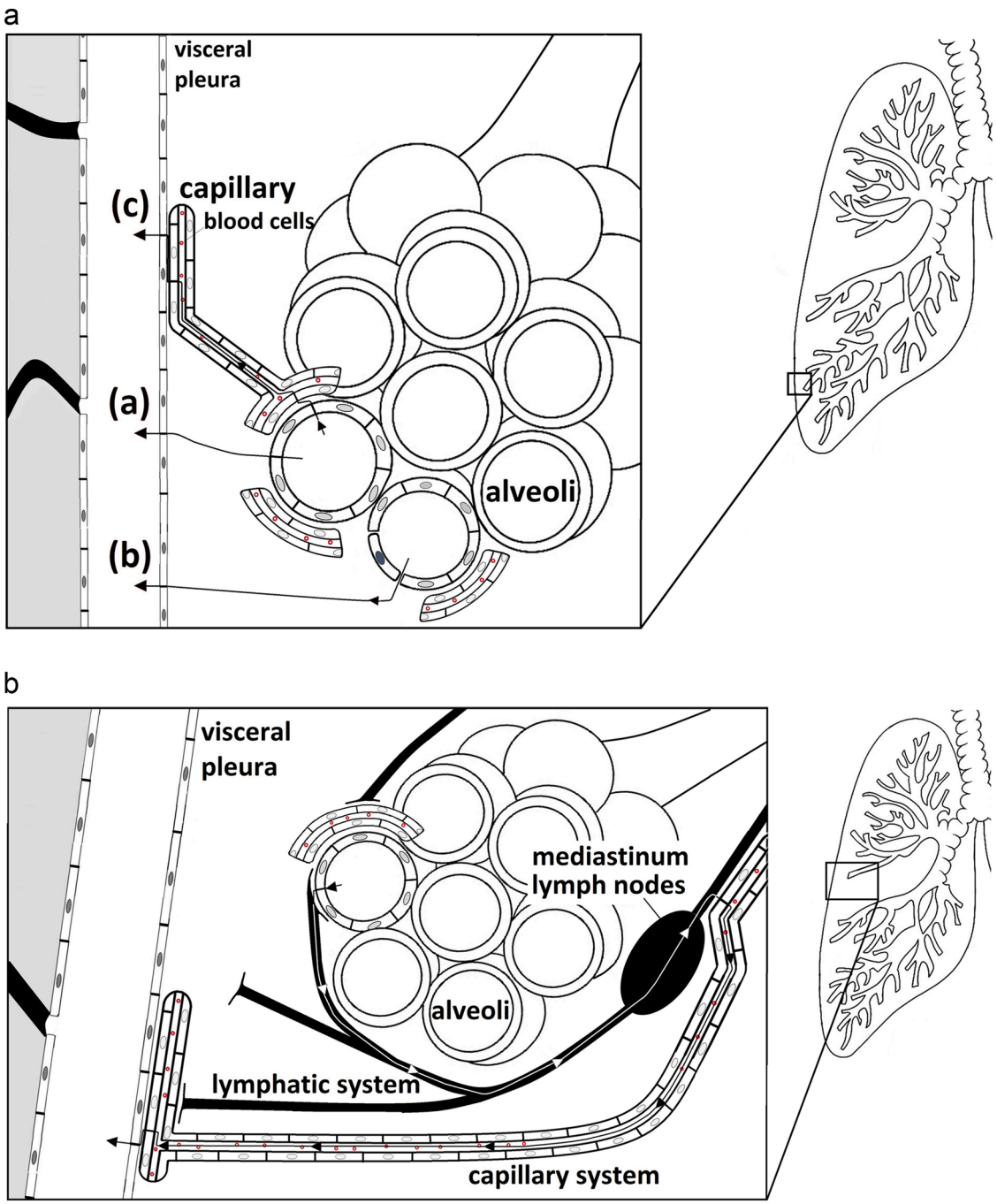

Fig. 3. (a). The major possible pathways for either free or "macrophage-engulfed fibres" translocation from the parenchyma (alveoli) to the pleural space. Path (**a**) the paracellular way, through the space between the cells; Path (**b**) the paracellular way, through the space between the cells that is widened by fenestration in the epithelial lining due to inflammation leading to the death of alveolar epithelial cells; Path (**c**) the "capillary way" where translocation occurs from the parenchyma to the capillaries into the blood torrent followed by extravasation in the pleural capillaries during the formation of pleural fluid. Fig. 3 (b). The fourth possible pathway (path (**d**)) for either free or macrophage-engulfed fibres' translocation from the parenchyma (alveoli) to the pleural space. The "lymphatic-capillary way" where translocation occurs from the parenchyma to lymph flow to the mediastinum and then into the blood via the thoracic duct followed by extravasation in the pleural capillaries.

pleural pressure are reversed, resulting in a net flow of fluid and fibres directly into the pleural space from the underlying parenchyma. Donaldson et al. (2010) cautioned that this suggested mechanism does not explain normal transit of particles to the pleura giving rise to black spots in individuals who do not have pulmonary inflammation.

(c) A mechanism similar to (a) or (b) but in which the fibre translocation occurs directly to capillaries into the blood stream via the thoracic duct, followed by extravasation into pleural capillaries during the formation of pleural fluid (path **(c)** in Fig. 3a).

(d) A mechanism similar to (c) in which translocation occurs by normal lymph flow centrally to the mediastinum and then into the blood via the thoracic duct, followed by extravasation into pleural capillaries during the formation of pleural fluid (path (**d**) in Fig. 3b). Donaldson et al. (2010) regarded this mechanism as a tortuous pathway which disregards the filtering role of lymph nodes.

3.3. Frustrated phagocytosis and inflammatory response

It was already said that if a fibre is biodurable, and its length is > 10 μm, macrophages cannot completely engulf it in the alveolar space giving rise to a process called "frustrated phagocytosis" (O'Neill, 2008; Donaldson et al., 2010). In failed attempts to dissolve longer fibre, macrophages may stimulate an inflammatory reaction with recruitment of other inflammatory cells such as neutrophils into the alveolar compartment. A as shown in Fig. 4, the inflammatory burst may prompt:

1. production and release of ROS like O_2^-, H_2O_2 and $HO^\bullet$ and reactive nitrogen species (RNS) like $^\bullet NO$ and $^\bullet ONOO$ (Dostert et al., 2008; Kamp, 2009). Excess ROS/RNS production is responsible for the molecular, cellular and tissue abnormalities falling under the umbrella termed "oxidative stress" (Faner et al., 2012);
2. release of (transforming or tumour) growth factors like TGF-β, responsible for the growth of epithelial cells;
3. activation of p53, a transcriptional activator modulating downstream target gene expression involved in DNA damage responses and cancer suppression, important in preventing the accumulation of gene mutations (Brady et al., 2011). p53 controls expression of genes involved in apoptosis (see below), cell cycle arrest, and DNA repair (Kamp and Weitzman, 1999). The response of p53 to DNA damage due to oxidative stress is to halt tissue injury and cancer progression by augmenting DNA repair and/or promoting apoptosis of cells with DNA damage that overwhelms the repair mechanisms (Harper and Elledge, 2007);
4. activation of the Nalp3 inflammasome (Kelley et al., 2019). Inflammasomes are cytoplasmic protein complexes activated upon recognition of a number of diverse danger signals whose subsequent activation leads to interleukin IL-1β secretion (see below). Inflammasome activation is triggered by ROS generated by a NADPH oxidase, the reduced form of nicotinamide adenine dinucleotide phosphate (Dostert et al., 2008);
5. release of cytokine proteins like interleukin IL-1 β, activated as a proprotein, which is proteolytically converted to its active form by the enzyme caspase-1. IL-1 β is involved in a variety of cellular activities, including cell proliferation, differentiation, and most of all, apoptosis;
6. the release of cytokines like TNF-α. TNF-α in turn alerts other cells of the immune system and stimulates the production of chemokines (like IP-10, MCP-1, and RANTES) involved as mediators in the early inflammatory response (Driscoll et al., 1997; Hillegass et al., 2013; Comar et al., 2016);
7. the upregulation of transcription factors such as activator protein-1 (AP-1) and nuclear factor-κB (NF-κB);
8. the production of receptor tyrosine kinases (RTKs), high-affinity cell surface receptors playing a critical role in the development of lung cancer and mesothelioma. RTKs specifically phosphorylate tyrosine amino acids tyrosine-kinases and regulate the growth of fibroblasts (collagen deposition).

The release of all the above pro-inflammatory and inflammatory molecules/substances (with special attention to Nalp3 inflammasome, TNF-α, and NF-κB) can be chronic, especially when the inflammatory action is prompted by biodurable fibres like crocidolite and erionite, and involves the continuous recruitment of phagocytes sustaining the chronic inflammatory process (Gaudino et al., 2020). As described below, chronic inflammation is known to play a key role in pathogenesis/carcinogenesis because at the site of inflammation, mesothelial cells may avoid programmed cell death and undergo oncogenic transformations (Carbone and Yang, 2017). The surviving mutated cells continue to proliferate and accumulate genetic mutations, leading to both cancer in the lungs and mesothelioma in the pleura (Gaudino et al., 2020). During the pro-inflammatory activity, an overexpression of immunosuppressive mediators (cytokines TGF-β and IL-10) mediated by T limphocytes, M2–polarized macrophages and accumulation of potent immunosuppressive MDSC is also observed (Huaux, 2018). A number of physical-chemical fibre parameters play a role in determining the inflammatory action and the activity for immunosuppression (Gualtieri, 2021): fibre length determines local chronic inflammation due to AM frustrated phagocytosis; hydrophobic character of the fibre surface influences cell uptake (Gualtieri et al., 2017), the viability of AM phagocytosis and local chronic inflammation; fibres' surface area rules the overall size of the fibre and consequently AM frustrated phagocytosis and local chronic inflammation; the content and release of metals (especially iron) from the fibres prompt direct production of ROS/RNS and cause local chronic inflammation; the fibre dissolution rate regulates the persistence at site of deposition, triggering chronic inflammatory activity; the fibre zeta potential and fibres' aggregation rule the production of ROS/RNS at the fibre surface causing local chronic inflammation. In each section dedicated to crocidolite, chrysotile and

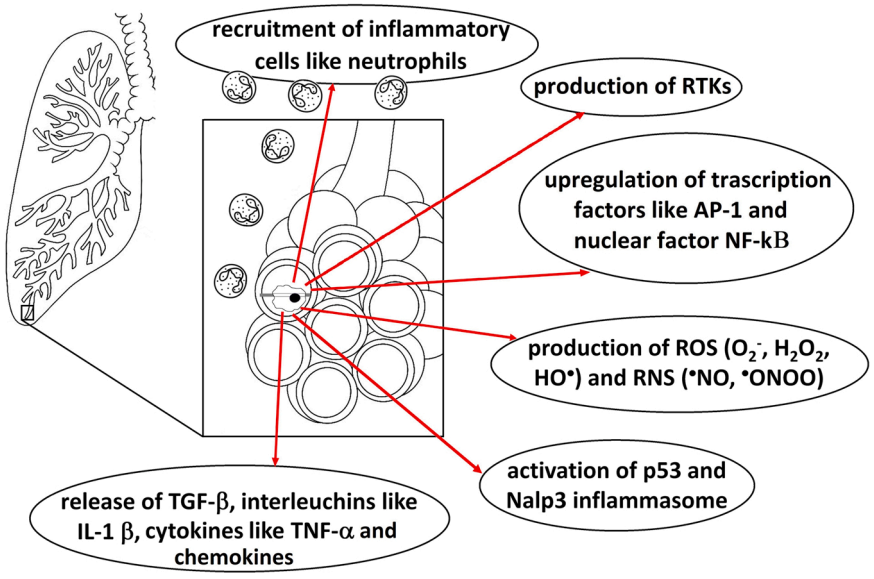

Fig. 4. The cascade of biochemical processes ignited by the inflammatory burst during the frustrated phagocytosis of the alveolar macrophage (AM) in the attempt to dissolve the engulfed fibre.

erionite the inflammatory activity will be tentatively discussed with respect to the mineralogical nature of the fibres.

The inflammatory burst anticipates the death of the macrophage through three processes:

1. apoptosis: programmed ATP-regulated cell death, beneficial for the organism because cells with extensive DNA damage are eliminated without eliciting further inflammatory response. Cell apoptosis dampens the inflammatory burst because anti-inflammatory cell surface molecules that trigger immediate anti-inflammatory signalling pathways are released (Szondy et al., 2017);
2. ferroptosis: an oxidative iron-dependent (Toyokuni, 2019) type of programmed cell death genetically and biochemically distinct from apoptosis. According to Cao and Dixon (2016), how iron promotes ferroptosis remains unclear. However, it is possible to sketch a general pattern: an overload of intracellular Fe^{2+} triggers the donation of electrons to the oxygen atoms with production of ROS leading to the accumulation of toxic lipid ROS (L-ROS). The accumulation of these lipid peroxide molecules in turn causes unrestricted lipid peroxidation, subsequent membrane damage and the death of the cell. This mechanism is possible because a number of bio-chemical defence processes are inhibited or switched off: (i) the action of intracellular iron chelators is inhibited (Cao and Dixon, 2016); (ii) the amino acid antiporter system x_C^- or activation of the iron transporters transferrin and lactotransferrin regulating the intracellular iron load and extracellular transfer are inhibited (Tang and Kroemer, 2020); (iii) the expression or activity of intracellular antioxidant enzymes, such as glutathione peroxidase 4 (GPX4) is blocked (Tang and Kroemer, 2020);
3. necrosis: the premature cell death by autolysis which, unlike apoptosis, is always detrimental for the organism. Necrosis does not follow the apoptotic signal transduction pathway. Instead, various receptors are activated resulting in loss of cell membrane integrity and the release of products like ROS and RNS into the extracellular space. This prompts a second inflammation burst which attracts other phagocytic cells in the attempt to eliminate the necrotic cell by phagocytosis.

During and after the occurrence of frustrated phagocytosis, both the fibre and necrotic cell cause the release of ROS and RNS into the extracellular medium. This causes cytotoxic and genotoxic damage to nearby alveolar cells and fibroblasts. ROS production is called "primary" if it is elicited at the surface of the fibre in contact with the cells, or "secondary" if prompted by the cells' inflammatory activity. Primary ROS production at fibre surfaces is mediated by metals, specifically iron (Fubini et al., 1995; Turci et al., 2017). Surface iron-mediated primary ROS production occurs via the Fenton-catalysed Haber-Weiss reaction (Hardy and Aust, 1995; Fenoglio et al., 2001; Turci et al., 2011): $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + {}^{\bullet}OH$. Fe^{2+} is also generated by the reduction of Fe^{3+} by ascorbate, cysteine, glutathione or other cellular reductants.

Activated macrophages are also capable of reducing Fe^{3+} because they release $O_2^{\bullet-}$ (Hardy and Aust, 1995). Hence, production of ROS may also take place in the presence of Fe^{3+}, which is known to be reduced by H_2O_2 to Fe^{2+}, according to the reaction: $Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + {}^{\bullet}O_2^- + 2\,H^+$ (Turci et al., 2011; Gualtieri et al., 2019a). Both surface Fe^{3+} and Fe^{2+} also prompt the cleavage of the C-H bond in the formate ion: $H\text{-}CO_2^- \rightarrow {}^{\bullet}CO_2^- + H^+ + e^-$. This reaction, the mechanism of which is still partially obscure, may occur with several molecules of biological interest such as peptides, proteins and lipids (Turci et al., 2011).

Hardy and Aust (1995) made an important point on the relationship between primary ROS production and DNA damage. Generation of primary hydroxyl radicals on the surface of the fibres is only important in reactions with biomolecules when the fibre is within approximately 10 Å of the target biomolecule, because of the diffusion-controlled reaction kinetics of hydroxyl radicals. Hence, DNA damage from primary hydroxyl radicals can only occur when the fibre is in the nucleus of the cell. Because fibres are seldom observed in the nucleus, the role of primary hydroxyl radicals formed at the surface of the fibre in inducing DNA damage should be reconsidered. On the other hand, if iron can be mobilized from the fibres by chelators, such as citrate, the redox activity might be altered, and the chelate complex could diffuse throughout the cell and have the potential of catalysing the formation of hydroxyl radicals to damage DNA (Hardy and Aust, 1995).

ROS production is accompanied by lipid peroxidation that exerts a strong toxic effect. Lipid peroxidation may change the assembly, composition, structure, and dynamics of cell membranes. Lipid peroxides, as other reactive compounds, are capable of inducing further ROS production or degrading into reactive compounds capable of cross-linking DNA and proteins (Gaschler and Stockwell, 2017). If the process of frustrated phagocytosis in the lung environment with subsequent cell necrosis and inflammation activity is chronic, DNA damage of the cells will also be chronic, resulting in DNA and/or epigenetic mutations leading to uncontrolled proliferation of the mutated cells that initiate lung cancer (Mossman et al., 1996; Mossman and Gualtieri, 2020). Specifically, in the alveolar space and broncho-alveolar duct junction alveolar type 2 cells (AT2) are confirmed cell origin for lung adenocarcinoma and squamous carcinoma; in the broncho-alveolar duct junction, broncho-alveolar stem cells are also suspected to give rise to lung adenocarcinoma; in the bronchial epithelium, basal, broncho-alveolar stem, ciliated and goblet cells may give rise lung adenocarcinoma while neuroendocrine cells can be the origin of lung carcinoma (Sainz de Aja et al., 2021). The onset of lung cancers is associated with overexpression of epidermal growth factor receptors that belong to the ErbB family of tyrosine kinases (ERBB1 and ERBB2) due to mutations and/or chromosomal rearrangements. Amplification and/or mutations in the nuclear transcription factors MYC, MYB, JUN and FOS also occur in lung tumours, although their precise role in lung carcinogenesis is unknown (Fong et al., 2017). Regarding epigenetic mutations, different modes of epigenetic signalling have been recognized including DNA methylation, histone modifications, chromatin remodelling, and effects prompted by noncoding RNAs, a class of regulatory molecules that controls gene expression by binding to complementary sites on target messenger RNA (mRNA) transcripts (Mossman and Gualtieri, 2020). Among them, histone acetylation (addition of $-COCH_3$) and removal, i.e., via deacetylation and methylation, affect nucleosome-DNA interactions and alter gene expression. Aberrant DNA methylation, characterized by hypermethylation of CpG islands, and hypomethylation of other regions lead to silencing of tumour suppressor gene and/or genomic instability (Sandoval and Esteller, 2012).

3.4. Notes on lung fibrosis

Although the review is focused on lung cancer and mesothelioma, some considerations on interstitial pulmonary fibrosis are also made here. It is known that if cellular damage includes fibroblasts, an abnormal increase of collagen production leading to fibrosis may occur with excessive extracellular matrix protein deposition and activation of mesenchymal cells myofibroblasts (Cheresh et al., 2013). In mice, "asbestos" fibres have been seen to activate secondary ROS production by mitochondria and via AM-mediated H_2O_2 production by transferring electrons from complex III to Rac1. These reactive species drive down-stream signalling pathways, inflammation and cellular injury that result in "asbestosis" (Cheresh et al., 2013). ROS generated by "asbestos" fibres can also activate latent TGF-β, the most potent pro-fibrogenic multi-function protein found in nearly all fibrotic diseases (Cheresh et al., 2013).

According to Fasske (1986), in the process of pulmonary fibrosis induced by the presence of "asbestos" fibres in the alveoli, type II pneumocytes produce surfactant in excess, become necrotic and tubular myelin and lamellar bodies pass into the alveoli and the interstitium.

3.5. Formation of the Asbestos Bodies

Against the chronic detrimental inflammation activity in the lungs induced by repeated macrophage frustrated phagocytosis acts of long fibres, incapsulation of the fibres and formation of an iron-protein-mucopolysaccharide material called "asbestos or ferruginous bodies" (Roggli et al., 2004; Capella et al., 2017) is the ultimate defence mechanism of the body (Fig. 5). The coating process is mediated by iron with the ferritin core of "asbestos" body composed of ferric oxy-hydroxide FeOOH or $FeOOPO_3H_3$ if phosphate is present (Harrison et al., 1967). Besides iron and phosphorus, calcium and magnesium may also participate to the coating process (Pascolo et al., 2011). Incapsulation of the fibres in vivo is aimed at isolating them from the cellular medium and abating their bio-chemical activity in vivo. The influence of "asbestos" bodies on the cytotoxicity of "asbestos" fibres and the toxicity of iron associated with "asbestos" bodies are still open issues (Di Giuseppe et al., 2019). It is generally accepted that the coating of fibres to form ferruginous bodies is a protective mechanism. However, because the deposited iron appears to be redox active, it may actually contribute to the catalytic potential of the fibres (Lund et al., 1994). What is known so far is that only fibres with the Stanton-type size (length >10 μm) undergo incapsulation, a process mediated by iron (Koerten et al., 1990). Iron can be released during the inflammatory activity, if present in specific lysosomes, and can contribute to the formation of homogeneous aggregates of iron hydroxide ferritin or hemosiderin rich aggregates around the fibres (Di Giuseppe et al., 2019) showing a sub-spherical habit due to the globular shape of these iron-protein complexes (Richter, 1958).

4. Fibres in the pleural cavity

We have seen that single fibres or macrophage-engulfed fibre aggregates can be translocated from the alveoli to the pleural cavity where they are eventually cleared through the stomata in the lymphatic system. The lymphatic system does not contain red cells in the healthy state but contains a highly proteinaceous (albumin) fluid with prominent electrolytes (Na^+, K^+ and Ca^{2+}) and macrophages that can transport ingested fibres through the lymphatic torrent to the lymph nodes (Skinner et al., 1988).

Although the mechanism by which fibres are expelled from the lymphatic system (a suggested sketch is represented in Fig. 6) is one of many open questions, it has been postulated that particles from interstitial spaces and lymphatics may enter the airway lumen and be removed by mucociliary activity (Lippman et al., 1980).

Again, if the fibre is biodurable and its length is greater than 10 μm, frustrated phagocytosis by pleural macrophages, neutrophils and eventually modified mesothelial phagocytic cells may take place in the parietal pleura, because fibres cannot be completely engulfed by the phagocytic cells nor can they negotiate the stomata aperture. In support, Kane et al. (1986) observed that long "asbestos" fibres accumulated preferentially at the peritoneal face of the diaphragm near the stomata, hypothesizing that they were not expelled due to their length. The result is an aggregation of fibres at the stomata apertures forming so-called *black spots* (Fig. 7 modified after Boutin et al., 1996 and Donaldson et al., 2010). At these spots, just as in the mechanisms described above for alveolar macrophages in the lungs, frustrated phagocytosis by pleural macrophages may initiate an inflammatory burst prompting chemical signalling, production and release of the molecules, proteins and reactive species described for the alveoli and interstitium (Fig. 7, right). The inflammatory burst again precedes apoptosis, ferroptosis or necrosis of the macrophages.

The primary or secondary release of ROS and RNS in the pleural space may similarly cause cytogenetic and genotoxic damage to the nearby parietal pleural mesothelial cells. Secondary ROS production in the pleura also switches on signalling of mitogen-activated protein kinase MAPK, a type of protein kinase involved in directing cellular responses to a diverse array of stimuli, such as pro-inflammatory cytokines and activation of transcription factors other than AP-1 like the High Mobility Group Box 1 protein HMGB1 (Carbone and Yang, 2012).

Once again, if frustrated phagocytosis of indigestible fibres in the pleural space with subsequent cell necrosis and inflammation activity is chronic, chronic damage to the DNA of mesothelial cells by the pleural macrophages or by other mesothelial phagocytes will also occur. This chronic damage is responsible for DNA and/or epigenetic mutations leading to anomalous proliferation of the mutated cells, or the onset of MM.

Clinical studies have demonstrated that parietal but generally not visceral mesothelial cells are the targets of pleural MM. However, this would be the opposite for peritoneal mesothelioma due to the difference in lymphatic flow (Toyokuni, 2019). Although there are three subtypes of MM (epithelioid mesothelioma; sarcomatoid mesothelioma, including desmoplastic; biphasic mesothelioma), as well as rarer histological types, epithelioid MM represents the majority of all observed MMs (Skinner et al., 1988; Tsao et al., 2022).

Isolation of individual fibres through encapsulation within an "asbestos body" may occur in principle inside the pleural cavity, but actual observation of in situ formation of such bodies in the pleural space is very difficult. "Asbestos" bodies have been observed in the

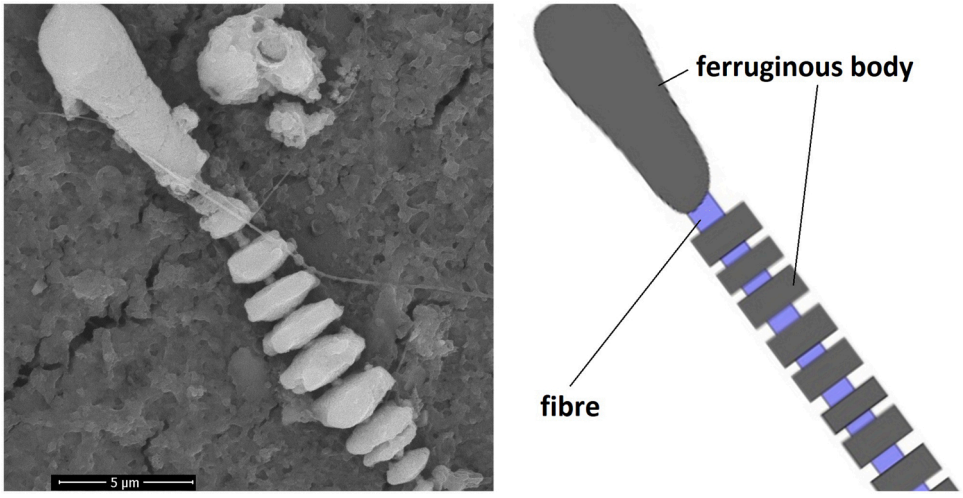

Fig. 5. A FEG-SEM image of a ferruginous bodies partially incapsulating an amphibole fibre found in the lungs of a subject diagnosed with mesothelioma (see the details in Di Giuseppe et al., 2019).

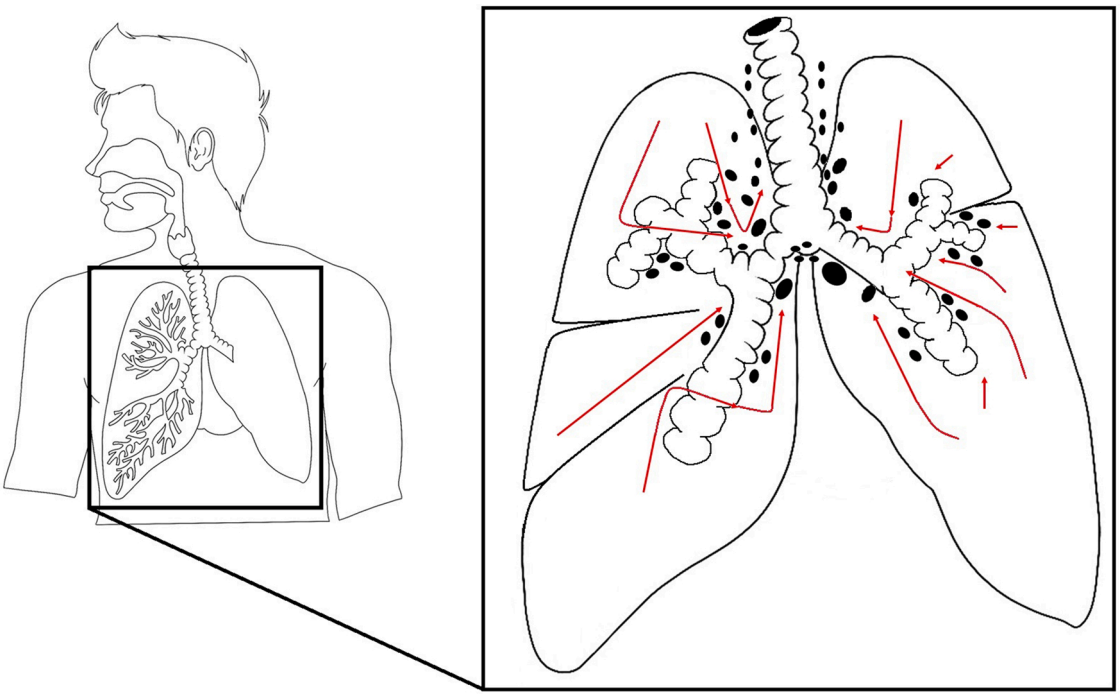

Fig. 6. A sketch of the lymphatic system in the chest area. Lymph nodes are depicted as black ellipses. Arrows indicate the major flow of the lymphatic system. Note the large size of the nodes at the mediastinum (mediastinal lymph nodes).

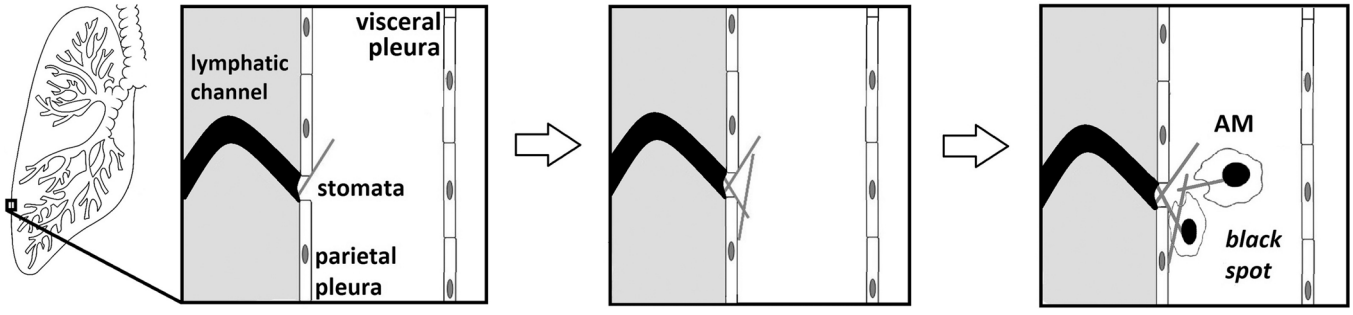

Fig. 7. Progressive aggregation of biodurable fibres at the stomata, the aperture of the pleura to the lympathic system, forming the so-called *black spots* where chronic frustrated phagocytosis by pleural macrophages occurs.
(modified after Donaldson et al., 2010).

pleural cavity (e.g. Pascolo et al., 2016), but it is impossible to know whether they were formed there or are a product of translocation from the lungs or other organs, with or without the carrier of macrophages. Translocation of "asbestos" bodies to organs other than the lungs has been widely reported in the literature (Auerbach et al., 1980). They have been reported in hilar and mediastinal nodes, in lymphatics, in the spleen, abdominal walls and in the peritoneum (Godwin and Jagatic, 1970).

According to Toyokuni (2019), "asbestos" fibres cause massive hemolysis, collecting iron from the surrounding environment, such as dead macrophages and disrupting red blood cells. In addition, carcinogenesis in parietal mesothelial cells concomitant with phagocytosis of iron-coated fibres has been suggested as promoted by adipocytes (Toyokuni, 2019). Hence, both the formation of "asbestos" bodies in situ in the pleural cavity and their contribution to the toxicity mechanisms in vivo are still debated issues.

5. The behaviour of crocidolite, chrysotile and erionite in the respiratory system

This section describes the behaviour of respirable fibres of crocidolite, chrysotile and erionite when they are inhaled and enter the respiratory system. These fibres are representative of three different classes of silicates: chain silicates (amphiboles), layer silicates (serpentine) and framework silicates (zeolites) (Fig. 8).

A description of the crystal-structure of these minerals is provided in Appendix II (Supplementary material).

The focus is on how the physical and crystal-chemical characteristics of the fibres influence the behaviour in vivo and the potency to induce lung cancer and mesothelioma. The description is aimed at comparing the basic behaviour of *single* fibres in vivo and is irrespective of exposure (Wylie, 1984) and dose rates (Barrett et al., 1989; Mossman et al., 1990) and other complementary synergetic factors like "asbestos"-related release of polycyclic aromatic hydrocarbons (PAH) (Kennedy and Little, 1974) and smoke (Keeling et al., 1993). It should be remarked that all these factors can affect each other and the effects of these cross-correlations are still poorly understood. For example, it is well

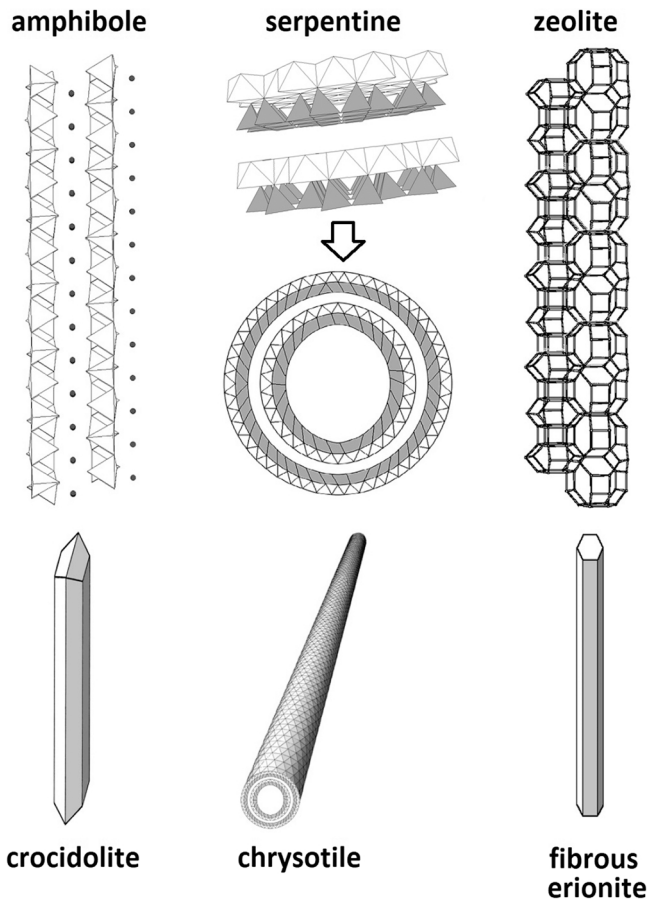

Fig. 8. The three classes of mineral fibres discussed in this work: crocidolite (amphibole "asbestos"), chrysotile (serpentine "asbestos") and erionite (zeolite).
(modified after Fig. 1, Gualtieri et al., 2019b).

known that there are limitations in understanding exposure-response relationships for various "asbestos" types and exposure levels and disease (Case et al., 2011).

This description is based on the body of existing literature data and original experimental data relative to three "real" mineral fibres (standard crocidolite South African, NB #4173–111–3, Balangero (Torino, Italy) chrysotile and Erionite-Na from Jersey, Nevada, USA) collected during the long-term research activity of the author.

A representative sample of UICC crocidolite standard (South African, NB #4173–111–3) is characterized by long fibres (mean length of 25 μm and diameter of 0.3 μm), prevailing "acicular" asbestiform crystal habit, high density (3.3 g/cm^3) due to the very high iron content (total Fe_2O_3 =17.87 wt % and FeO=17.42 wt%: Pacella et al., 2019), and biodurability (estimated total dissolution time of 66 years in acidic fluid simulating the intracellular phago-lysosome medium: Gualtieri et al., 2018).

A representative sample of chrysotile sample from the Balangero mine (Italy) has very long fibres (mean length of 35 μm and diameter of 1.5 μm) with a prevailing curled crystal habit (see also Loomis et al., 2010). Fibres have a low density (2.53 g/cm^3), low iron content (total Fe_2O_3 =0.4 wt% and FeO=2.5 wt%: Pollastri et al., 2016a), and are not biodurable (estimated total dissolution time of 3 months in acidic fluid simulating the intracellular phago-lysosome environment: Gualtieri et al., 2018).

A representative sample of Erionite-Na from Jersey (Nevada, USA) is characterized by short fibres (mean length of 12.5 μm and diameter of 0.5 μm) with an acicular crystal habit. Fibres have a low density (2.11 g/cm^3) and low iron content (total Fe_2O_3 =0.3 wt% and FeO=0.12 wt% due to iron-bearing impurities like amorphous iron-rich nanoparticles, micro-particles of iron oxides/hydroxides, and flakes of nontronite dispersed at the surface of the fibres: Gualtieri et al., 2016). Erionite fibres are highly biodurable (estimated total dissolution time of 181 years in acidic fluid simulating the environment of intracellular phago-lysosomes: Gualtieri et al., 2018) and display cation exchange capacity.

The complete mineralogical and chemical-physical characterization of these mineral fibres can be found in Pollastri et al., (2014, 2015).

5.1. *The case of crocidolite*

5.1.1. *Crocidolite through the upper respiratory tract*

The mucociliary escalator, the first defence mechanism of the human body, is not effective in filtering crocidolite fibres because of their crystal habit and density. "Acicular" fibres like crocidolite are less prone than curled ones (like those displayed by chrysotile) to be deposited in the upper bronchial or bronchiolar tracts (Harris and Timbrell, 1977) and have higher probability to deposit in the alveoli. The high density of the crocidolite fibres also favours their descent into the deep respiratory tract as depositional depth in the lungs is deeper for denser fibres (Yeh et al., 1976).

5.2. *Crocidolite in the deep respiratory tract*

Crocidolite fibres reaching the alveoli are subject to tentative proteins' detoxification and clearance by AM. Unfortunately, both defence mechanisms are frustrated. A protein corona or a protein coverage can be catalysed at the surface of the fibres but this phenomenon cannot be as significant as for NPs because of the considerable dimension of the fibres. Besides that, because crocidolite is biodurable, a successful alveolar clearance by macrophage phagocytosis is not possible for fibres longer than 10 μm.

Frustrated phagocytosis and subsequent cell death prompt inflammatory activity (Donaldson et al., 2010). During and after the frustrated-phagocytosis act, primary ROS production from the surface of the iron-rich crocidolite fibres (Martra et al., 1999) and secondary ROS production due to the cell inflammation activity are boosted, causing cyto- and geno-toxic damage and lipid peroxidation of the alveolar cells and fibroblasts. Recently, Pacella et al. (2021) showed that the alteration of crocidolite surface in lysosome simulated lung or body fluids (Innes et al., 2021) at pH= 4.5 promotes the occurrence of Fe centres in proximity of the fibre surface, particularly in the form of Fe^{2+}, of which the bulk is enriched with respect to the oxidized surface. This finding has been confirmed by Vigliaturo et al. (2022) for amosite fibres: once taken up by alveolar epithelial cells, the amphibole particles undergo surface incongruent dissolution with the grain boundary becoming progressively enriched in Fe. This process causes the exposure at the particle boundary of Fe^{2+}-containing structural sites and the erosion of the Fe^{3+}-rich outer layer. The surface erosion of crocidolite fibres may prompt release of iron atoms and eventually prompt the so-called 'Trojan horse effect' (Studer et al., 2010) invoked to explain the toxicity in vitro of chrysotile (see the section on the chrysotile case below).

Iron mediated primary ROS production at the surface of the crocidolite fibres which occurs via the Fenton-catalysed Haber-Weiss reaction is of paramount importance as the function of iron in inducing pathobiological effects in vivo is now universally accepted. In fact, the comparison of iron-free versus iron-doped fibres has confirmed the role of iron in toxicity relevant "asbestos" reactivity (Gazzano et al., 2007; Turci et al., 2011). A fibre that does not expose iron is non-reactive in terms of ROS generation and cellular damage. However, on the basis of the present and past studies, it was shown that even a very small amount of iron induces radical reactivity, cytotoxicity and genotoxicity (see the case of chrysotile). Any further increase in iron loading progressively decreases, instead of increasing, the reactivity.

Long crocidolite fibres also activate the epidermal growth factor receptor (EGFR) via direct membrane interactions or by affecting the kinetics of EGFR binding to its ligands (Pache et al., 1998). Being an iron-rich biodurable fibre (Gualtieri et al., 2018), the process of frustrated phagocytosis in the alveolar space, subsequent cell necrosis and inflammation activity as well as the primary ROS production due to the iron activity at the surface of the fibre are chronic. Because crocidolite fibres are stable both in extracellular and intracellular media, they are sort of an iron storage of unlimited capacity for the primary ROS production. Moreover, after inhalation of crocidolite "asbestos", cell-specific increases in unphosphorylated and phosphorylated ERK1 and ERK2 were observed in bronchiolar and alveolar type II epithelial cells in areas of epithelial cell hyperplasia (Cummins et al., 2003). These bio-chemical processes can cause chronic damage and/or mutation of the DNA of the alveolar cells leading to uncontrolled proliferation and eventually lung cancer. In fact, exposure to crocidolite is related to the incidence of adenocarcinoma of the lung, squamous cell lung cancer, and undifferentiated large cell lung cancer among the workers at the Australian crocidolite mine of Wittenoom (de Klerk et al., 1996).

Crocidolite causes fibrosis when cell damage and mutation of fibroblasts result in an abnormal collagen production. The findings of Adamson and Bowden (1987) for rats exposed to crocidolite fibres suggest that injury to bronchial and bronchiolar epithelium allows long crocidolite fibres to reach the *interstitium* where subsequent macrophage-fibroblast interactions result in a severe fibrotic reaction that resembles the bronchiolar component of human "asbestosis". According to Shukla et al. (2003), crocidolite is among the most potent mineral fibres for inducing fibrosis and causes preferential mtDNA damage rather than nuclear DNA damage.

Indigestible crocidolite fibres longer than 10 μm can be incapsulated by "asbestos" bodies that represent a successful attempt by the host to sequester the metal adsorbed to the surface of a fibre and diminish its oxidative power. Although the overall mechanism is well explained, whether iron-rich fibres like crocidolite catalyse and/or contribute to the nucleation and growth of "asbestos" bodies is still unclear. Apparently, literature data rule out any relationship between the amount iron of the "asbestos" fibres and the formation of the "asbestos" bodies indicating that iron is biogenic and released during the inflammatory activity by the engulfing macrophages (see for example: Botham and Holt, 1971). Macrophages have been proposed to be the source of iron in ferruginous body formation and responsible for the deposition of iron on phagocytized fibres that have a high affinity for iron (Hardy and Aust, 1995). An earlier work by Botham and Holt (1971) concluded that the source of the metal that accumulates during the formation of a body is not from the fibre. Later, Koerten et al. (1990) claimed that, irrespective of the chemical nature of the long fibre and especially the iron content, when an iron compound is present in the lysosomes, an "asbestos" body starts to precipitate. For these authors, "asbestos" body formation is dependent on the content of the lysosomes and is therefore a coincidental phenomenon. Bursi Gandolfi et al. (2016) also found that the content of Fe of the fibres apparently does not govern the amount of coated fibres. Notwithstanding, coordination of host iron is more likely to account for iron concentrations. Increases in concentrations of host iron could possibly promote its mobilization, elevated ferritin expression, and result in higher rates of "asbestos" body formation (Ghio et al., 1997). Ghio et al. (2004) also postulated that iron is accumulated onto crocidolite fibres in the lungs of guinea pigs mostly in the form of ferritin and not chemically reactive in oxidant production. Whether or not fibres make iron available to the nucleation and growth of these corpuscles, the contribution of biodurable crocidolite should be minor to null. The scenario may eventually change in the case of chrysotile that releases iron during early dissolution in contact with the lysosomal vacuoles (see the section below dedicated to chrysotile).

5.3. Crocidolite in the pleural cavity

Although it was remarked that the mechanisms of fibre post-depositional movement (translocation) are still obscure, naked fibres as well as macrophage-engulfed fibres can be eventually translocated from the alveoli to the pleural cavity where they are possibly cleared through the stomata in the lymphatic system. The so-called "pleural drift" (Coin et al., 1992) should be more difficult for long fibres. In studies of rats exposed to "asbestos" fibres, Coin et al. (1992) found no evidence of chrysotile fibres translocation to the peripheral areas whereas Morgan et al. (1977) observed sub-pleural concentrations of crocidolite. Although there were differences in the times of follow-up of their studies, the different dynamics of fibres' pleural drift may be explained by the different biodurability and by the different crystal habit of crocidolite and chrysotile with "acicular" crocidolite fibres that are more easily translocated than curled chrysotile fibres.

If they accomplish the pleural drift, long crocidolite fibres undergo frustrated phagocytosis by pleural macrophages in the parietal pleura because fibres cannot be completely engulfed by the phagocytic cells nor can negotiate the stomata aperture. Fibres gather at the stomata apertures and, following the scenario depicted for the alveolar space, frustrated phagocytosis of the pleural macrophages and phagocytic mesothelial cells ignite the inflammatory burst. Again, primary and/or secondary release of ROS and RNS causes cyto- and geno-toxic damage of the parietal pleural mesothelial cells. This inflammatory process is chronic in the case of indigestible crocidolite fibres with a prolonged DNA damage of the mesothelial cells eliciting DNA and/or epigenetic mutations and eventually abnormal proliferation of the mutated cells resulting in the onset of MM. Hence, it is not surprising that crocidolite is considered the most potent fibre type with respect to the pathogenesis of MM (Schneider et al., 2008).

As seen above for the general case, incapsulation of crocidolite fibres inside "asbestos" bodies in principle may also occur in the pleural cavity although translocation of aggregates of indigestible crocidolite fibre-"asbestos" body from the lungs or other organs is widely reported in the literature (see for example, Auerbach et al., 1980). Given its biodurability, the occurrence of coated long crocidolite fibres in the pleural cavity should be frequent and certainly more likely than the case of chrysotile bodies.

It was reported above that crocidolite is probably among the most potent mineral fibres for inducing fibrosis in the lungs (Shukla et al., 2003) with its fibrogenic action that is expected to exert even in the pleura. Stephens et al. (1987) measured high amphibole (crocidolite and amosite) counts in cases of patients with pleural fibrosis, with values ranging from 2 to 4–28 $\times$ 10^6/g dried lung in the pleura. Recently, Bernstein (2015) demonstrated that crocidolite produced a rapid inflammatory response in the lung parenchyma and the pleura of exposed rats, inducing a significant increase in fibrotic response in both of these compartments. In that study, Bernstein (2015) also quantified the evolution of fibrosis in response to the short-term inhalation of crocidolite versus chrysotile-containing brake-dust. An increase in fibrotic response of the visceral pleural wall to 200 % that of the air control at 365 d post exposure and a concomitant inflammatory response in the pleural cavity with the development of the fibrotic response in the pleural walls were observed for crocidolite but not for the chrysotile rich dust.

5.4. The case of chrysotile

5.4.1. Chrysotile through the upper respiratory tract

Chrysotile also displays considerable alveolar deposition. However, clearance of chrysotile in the upper respiratory system should be more efficient than that of crocidolite because of the curled nature of the chrysotile fibres settling by impaction in the upper airway bifurcations (Harris and Timbrell, 1977) where they are cleared by the mucociliary elevator (Evans et al., 1973). Moreover, the lower density of chrysotile fibres with respect to crocidolite limit their descent into the deep

respiratory tract.

5.5. Chrysotile in the deep respiratory tract

Even for the chrysotile case, a full protein coverage at the surface of the fibres is unlikely but chemical/physical dissolution by AM is more efficient with respect to crocidolite because chrysotile is not biodurable (Hume and Rimstidt, 1992a,b). Hence, successful clearance of fibres shorter than 10 μm is accomplished via chemical-physical dissolution during AM phagocytosis while longer chrysotile fibres are subject to partial phagocytosis (Donaldson et al., 2010) but fall apart in the lung into shorter fibres when attacked by the acid environment of the macrophage lysosome vacuoles (Bernstein et al., 2013). To a first approximation, this model tells us that chrysotile is less toxic and pathogenic in the lung environment than crocidolite. Notwithstanding, another effect may be invoked to cause a breach in the wall of the 'fibre toxicity paradigm' and to explain some toxicity effects observed in vitro for chrysotile. This alternative model is based on the results of in vitro studies (see for example, Gualtieri et al., 2019b) and requires further experimental validation because static in vitro studies like those performed by Gualtieri et al. (2019b) may have limitations, the dose of the fibres is not calculated and there is still no direct evidence in vivo of this effect. Said that, chrysotile fast dissolution (due to the acidic environment created by the lysosome sacs) prompts the release of the metals hosted in its structure (bioavailability) in the lung environment (Fig. 9), mimicking the so-called 'Trojan horse effect' known to explain the toxicity of nanoparticles (Studer et al., 2010; Innes et al., 2021). This connection also needs confirmation as the study of Studer et al. (2010) on nanoparticles was not performed in vivo and did not report the metal release per particle from the nanoparticles used.

Certainly, iron is the prevailing metal released from the brucite octahedral sheet of chrysotile (Pollastri et al., 2016b) and even very small amounts of iron are known to induce radical reactivity, cytotoxicity and genotoxicity in vitro (Turci et al., 2011).

Besides releasing their toxic cargo both intra-cellularly and extracellularly, corroded chrysotile fibres leave behind a silica-rich amorphous structure that maintains the original fibrous habit (*pseudo-morphosis*). Chrysotile silica pseudo-morphs with their distorted surface silanol groups continue to induce frustrated phagocytosis and cell disturbance, and although to a lower extent, they still preserve a reduced but significant ROS production (Gualtieri et al., 2019c).

Hardy and Aust (1995) observed that silicate surface groups (especially silanol groups) dissipate shortly in the moist environment of the lungs and may eventually be involved in acute biological effects. This model also explains why metal-ion chelators such as ethylenediaminetetraacetic acid, as well as iron-complexing agents such as desferrioxamine were found to inhibit chrysotile-induced lipid peroxidation and in vitro cell damage (Kandaswami et al., 1988). This

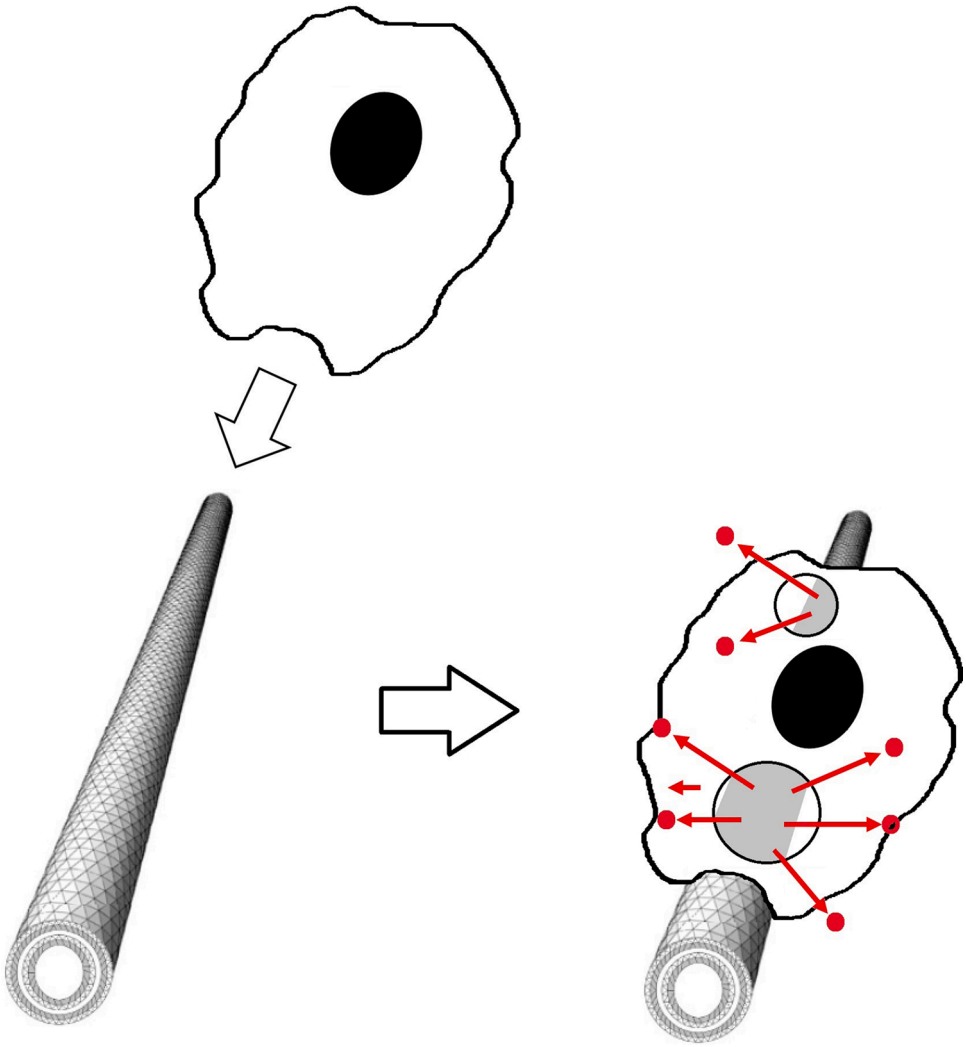

Fig. 9. Acidic lysosome vacuoles during the AM phagocytosis of a chrysotile fibre prompts the dissolution of the brucite sheet and release of the hosted metals in both the cytosol and eventually the extracellular environment, mimicking the so-called 'Trojan horse effect' observed during the engulfment for nanoparticles.

dissolution process occurs within a time span of weeks to months and hence should be regarded as acute to early-chronic toxic action. This toxic action differs from that of biodurable crocidolite inducing a chronic life-long detrimental action (Gualtieri et al., 2019c).

As we have seen above, the residues of phagocytosis can be expelled (exocytosis) in situ. Alternatively, macrophages drift to the pleural cavity where they release their cargo through the diaphragmatic stomata.

During partial phagocytosis of a long chrysotile fibre, the macrophage dies and ignites an inflammatory burst. It should be said that this model is based on high dose in vitro studies but not directly observed in vivo at inhalation exposure concentrations a few orders of magnitude higher than past human exposures (Bernstein, 2022). Both the fibre and the metals released during its dissolution process induce primary ROS and RNS production. At the same time, the necrotic cell releases secondary ROS and RNS. These reactive species spread in the extracellular medium causing cyto- and geno-toxic damage of the nearby alveolar cells and fibroblasts. Because of the release of metals during dissolution, primary production of reactive species is intense and short-lived for chrysotile with respect to crocidolite. Secondary production of reactive species by AM partial phagocytosis of the fibrous silica residues of leached chrysotile also works on a short-medium term. On the other hand, indigestible crocidolite fibres are likely to prompt less intense but chronic events of primary and secondary production of reactive species. The difference in the intensity of the cyto-toxic action of chrysotile versus crocidolite in the short term may explain why COMET assay for DNA strand breaks revealed that chrysotile was most damaging to cells at equal weight concentrations when compared to amphibole types of "asbestos" (Mossman and Gualtieri, 2020). Furthermore, Craighead et al. (1980) and Mossman et al. (2011) showed that chrysotile is more cytotoxic than crocidolite or amosite on an equal mass or fibre concentration basis in rodent and human lung epithelial and mesothelial cells.

This mode of action is a key issue to explain the difference in the toxicity and pathogenicity of the two fibres but the model leaves an open question: is the probability of cell DNA damage higher for intense acute events (chrysotile) or for mild but chronic events (crocidolite)?

All other things being equal, if we assume the sequence of patho-biological events leading to lung cancer (cell DNA damage → cell DNA mutations → uncontrolled proliferation of the mutated cells: Mossman et al., 1996) and that chronic inflammation is a requisite for the onset of lung cancer (Kamp, 2009), a single fibre of chrysotile should have a lower probability to onset lung cancer than a single fibre of crocidolite.

Although the dose of exposure is not considered in this review, it can be speculated that a chronic long-term exposure to non-biodurable chrysotile fibres as in a working environment has comparable detrimental effects than a chronic exposure to a single or few biodurable crocidolite fibres. This assumption requires further evidence as it is mostly based on in vitro studies in which the exposures per cell are generally far greater than would occur in humans. In agreement, epidemiological studies showed that the risk differential for lung cancer between chrysotile and the two amphibole fibres crocidolite and amosite is between 1:10 and 1:50 (Hodgson and Darnton, 2010). This difference has also been remarked in the outcome of the recent quantitative modelling published by Korchevskiy and Wylie (see for example, Korchevskiy and Wylie, 2022; Wylie et al., 2022) where size parameters and morphological habits of elongate mineral particles are addressed as the main drivers for the observable difference in cancer potency. According to Bernstein et al. (2013), based on well conducted in vivo inhalation studies, the risk of an adverse outcome (lung cancer) may be low with even high exposures (to chrysotile) experienced over a short duration. Despite this body of scientific evidences, there are strong oppositions to this model based largely on in vitro studies with very high doses per cell. Given that workers are commonly exposed to a mixture of fibres, Stayner et al. (1996) suggested to apply the precautionary principle and treat chrysotile with virtually the same level of concern as crocidolite. As only chrysotile is used today this approach is apparently no longer justified.

Chrysotile can also cause fibrosis as it induces TGF-β and other pro-inflammatory/fibrotic signalling (Yee et al., 2008). It should be remarked that the work of Yee et al. (2008) is based on fibres instilled intratracheally into lung-specific dominant-negative p53 (dnp53) mice and that these authors concluded that benzo(a)pyrene (BaP) and the combination of BaP and chrysotile are potent inducers of adenocarcinoma in dnp53 mice.

Said that the potency of chrysotile of inducing fibrosis with respect to that of amphibole "asbestos" is still a matter of debate, the question is again whether activation of loci of fibrosis is more probable for intense acute events (chrysotile) or for mild but chronic events (crocidolite). Chronic long-term exposure to chrysotile fibres is expected to increase such probability. The testing of Coin et al. (1996), despite these authors used exposure concentrations greater than 50,000 ff/cm^3, seems to confirm this model. When compared with a single exposure, the triple exposure caused an enhanced inflammatory response as well as a prolonged period of increased DNA synthesis in the proximal alveolar region with hyperplastic fibrotic lesions subsequently developed in the same region persisting for at least 6 months after exposure (Coin et al., 1996). The scenario is still highly debated though with the concentration and size of the fibres that must be considered when assessing a potential risk (Mossman et al., 1990). Following a study on chrysotile miners, Churg et al. (1989) postulated that the degree of pulmonary fibrosis reflects fibre concentration. Nevertheless, contrary to predictions from animal studies, their results suggested that short fibres may be more important in the genesis of pulmonary fibrosis than is commonly believed. The same authors concluded that, in a population of heavily exposed chrysotile miners and millers, the presence of airways fibrosis reflects high tremolite "asbestos" burden. Whether chrysotile fibres themselves play a role in disease induction remains uncertain (Churg et al., 1993). Bernstein et al. (2018) found that in chrysotile exposure groups of rats, only a slight interstitial inflammatory response was observed with no peribronchiolar inflammation and no interstitial fibrosis. On the other hand, in the crocidolite exposure groups, the longer fibres once deposited in the lung did not clear and were observed in proximity to the visceral pleura and on the parietal pleura. Crocidolite produced inflammatory response progressed to Wagner grade 4 and interstitial fibrosis persisted following cessation of exposure.

Formation of "asbestos" bodies to "quarantine" chrysotile fibres in the lungs has been observed in both animal tests (see for example, Bursi Gandolfi et al., 2016, following intraperitoneal or intrapleural injection of a single 25 mg injection of mineral fibres in 1 ml of H_2O) and epidemiological studies (Churg and Warnock, 1981; Roggli, 2014; Capella et al., 2017). Because chrysotile with in vitro studies has been shown to releases iron during early dissolution in the lysosomal sacs created by partial phagocytosis of AM, these iron ions may act as active pool and co-factor for the growth of iron hydroxides forming the coating but not as key factor for the nucleation of the AB (Bursi Gandolfi et al., 2016). This effect has not been shown following inhalation exposure. There is agreement in the literature that the fibres' inorganic source of iron to the "asbestos" bodies is negligible. An early study by Suzuki and Churg (1969) indicated that the process of formation of "asbestos" bodies is basically similar for chrysotile and the iron-rich amosite and seemed of cellular origin. Pooley (1972) also claimed that the material forming the "asbestos" bodies is definitely biological in origin and corresponds closely to hemosiderin. The works by Roggli (2014) and Di Giuseppe et al. (2019) confirmed this model although Roggli (2014) in its review of epidemiological studies reported a predominance of amphibole asbestos bodies over chrysotile with chrysotile asbestos bodies that account only for about 2 % of all asbestos bodies that have been analysed by their laboratory. This is apparently in contradiction with earlier observations that the bulk of asbestos used commercially is chrysotile but it is not if one considers that chrysotile is not biodurable and dissolves much faster than amphiboles.

5.6. Chrysotile in the pleural cavity

Because of their low biodurability (chemical factor) and curled shape (physical factor), the "pleural drift" seems to be more difficult for chrysotile fibres than for crocidolite fibres. Hence it is possible to share the view that chrysotile has lower probability to reach the pleura (Bernstein et al., 2004). Several studies in the literature are in concert with this model and report that the number of amphibole fibres in the pleura is greater than the number of chrysotile fibres. For example, Boutin et al. (1996) reported that, although the distribution of "asbestos" fibres in the pleura was heterogeneous and concentrated in certain areas ("black spots") of the parietal pleura, in thoracoscopic biopsy samples from these black spots and from normal areas of the parietal pleura and lung from 14 subjects, amphibole fibres invariably outnumbered chrysotile fibres in all samples. This model has paramount consequences for the prediction of mesothelioma potency of these fibres because if we assume that a chrysotile fibre has low probability to reach the pleura, its potency for inducing MM also must be scaled with respect to a biodurable amphibole fibres. As seen above, this model explains why crocidolite is considered the most potent fibre type in inducing MM (Schneider et al., 2008). It is important to clarify that a lower mesothelioma potency does not mean that chrysotile cannot induce MM. Based on a review which did not differentiate co-exposure with amphibole asbestos, Kanarek (2011) stated that chrysotile causes mesothelioma worldwide. As a matter of fact, the mesothelioma potency of chrysotile versus crocidolite is probably the most debated issue in "asbestos" matter (Churg, 1988). On one side, there is a body of epidemiological evidences supporting the model that chrysotile mesothelioma potency is much lower than crocidolite mesothelioma potency (McDonald et al., 1980; Peto, 1980; Hodgson and Darnton, 2010) and that the risk of pleural mesothelioma in humans is deemed to be negligible for exposures to airborne chrysotile not contaminated by amphibole (Yarborough, 2007). In this regard, Garabrant and Pastula (2018) found values of mesothelioma potency index of 0.0012 for chrysotile, 0.099 for amosite, and 0.451 for crocidolite with a relative potency of chrysotile:amosite:crocidolite of 1:83:376. These figures have been recently confirmed by observed and modelled mesothelioma potency factor (R_M) reported by Korchevskiy et al. (2019) and following papers (Korchevskiy and Wylie, 2021; Wylie et al., 2022). On the other side, chrysotile has caused (Kanarek, 2011) and still causes mesothelioma worldwide. Smith and Wright (1996), despite their study did not provide an assessment of co-exposure to amphibole, have claimed that "*examination of all pertinent studies makes it clear that chrysotile is similar in potency to amphibole "asbestos". Since "asbestos" is the major cause of mesothelioma, and chrysotile constitutes 95 % of all "asbestos" use worldwide, it can be concluded that chrysotile is the main cause of pleural mesothelioma in humans*".

If chrysotile eventually reaches the pleura, it may cause intense but probably not chronic cyto- and geno-toxic in the short-medium term. This difference in the behaviour of chrysotile and crocidolite in vivo has been observed in rats. Bignon and Jaurand (1983) found that in rats whose pleural cavity had been separately injected with chrysotile and crocidolite fibres, chrysotile was immediately reactive in inducing inflammation and subsequently cancer, whereas crocidolite needed some in vivo modification to become inflammatory and carcinogenic.

As described earlier, the process of isolation of the fibres via incapsulation inside "asbestos" bodies in principle should also occur inside the pleural cavity. Although it is hard to prove experimentally, it is realistic to assume that a lower probability of occurrence of chrysotile fibres in the pleura results in a lower probability of occurrence of chrysotile-encapsulating "asbestos" bodies. This is still an unexplored research field and direct clear evidences linking the occurrence of chrysotile-encapsulating "asbestos" bodies in the pleura and related pleural diseases are lacking. In general, Tossavainen (2010) underlined that research needs progress in the studies on the specificity of lesions of the pleura as markers of "asbestos" exposure, on the prognosis of diffuse pleural abnormalities, on improving ultrasound imaging of the pleura, and development of new digital imaging techniques for the investigation of "asbestos"-related diseases.

If a chrysotile fibre has low probability to reach the pleura and its potency for inducing MM is subordinate to that of a biodurable amphibole fibre (Bernstein et al., 2013), its potency for inducing pleural fibrosis should follow the same hierarchy. Following this hypothesis, epidemiological studies have shown that chrysotile causes less pleural fibrosis and mesothelioma when compared with other "asbestos" types (Baur et al., 2012, 2015). The supposed lower potency of chrysotile for inducing pleural fibrosis is another open issue that requires experimental evidence and a clarification of the contradictory body of data from the literature. As an example, Gibbs et al. (1991) reported that the pleura predominantly contained short chrysotile fibres and a minimal numbers of amphibole fibres.

5.7. The case of erionite

5.7.1. Erionite through the upper respiratory tract

The mucociliary escalator detains erionite fibres in the upper respiratory tract more efficiently than crocidolite fibres. In fact, despite the same acicular crystal habit of both fibre types, the mean diameter of erionite fibres is generally greater (Bursi Gandolfi et al., 2016) than the mean diameter of crocidolite and the density of erionite fibres is lower than that of crocidolite fibres. Comparison with chrysotile is more difficult. On one hand, acicular erionite fibres are more prone to bypass the upper bronchial/bronchiolar tracts than curled chrysotile fibres (Harris and Timbrell, 1977) but the lower density of the erionite fibres makes the descent into the deep respiratory tract less probable.

5.8. Erionite in the deep respiratory tract

As observed for the "asbestos" fibres, protein detoxification is unlikely. Biochemical-physical clearance by AM is not effective because erionite is biodurable. On the other hand, physical clearance is possible because erionite fibres are generally shorter than "asbestos" fibres. The peculiar chemical-physical nature of the erionite fibres results in a different behaviour during the phagocytosis process with respect to "asbestos". First of all, iron content is very low in erionite and is entirely associated to Fe^{3+}-rich impurities at the surface of the fibres (Gualtieri et al., 2016). If iron belongs to impurities, in principle it should not have a relevance in the toxicity mechanism of erionite fibres. Notwithstanding, iron rich nano-impurities and especially nontronite are not stable in acidic environment and can be dissolved during phagocytosis (Gualtieri et al., 2019b), contributing to the production of ROS (as recently observed by Di Giuseppe et al., 2022). The dissolution of the surface impurities may leave a residue of iron atoms (Fig. 10) at specific sites anchored to the surface windows (like the 6-membered rings) of the zeolite channels (Gualtieri et al., 2016). These isolated active sites can also be responsible for the primary production of ROS.

Secondly, erionite is a zeolite that may host toxic metals like As, Be and Pb (Gualtieri et al., 2016, 2019a). These metals can be released by cation exchange both in intracellular and extracellular environment (another example of the "Trojan horse effect") and contribute to the primary production of ROS. Primary and secondary ROS production due to frustrated phagocytosis of the indigestible erionite fibres cause cyto- and geno-toxic damage and lipid peroxidation of the alveolar cells and fibroblasts.

Thirdly, the zeolite erionite can also exchange its extra-framework cations (namely K^+, Na^+, Ca^{++}) in both intracellular and extracellular media. In lung lining fluid (extracellular environment), the content of cations is: K^+ 6–29 mM, Na^+ 82–132 mM, Ca^{++} 4 mM while in cytosol (intracellular environment) the content of cations is: K^+ 139–150 mM, Na^+ 12 mM, Ca^{++} 2×10^{-4} mM (Lodish et al., 1999; Innes et al., 2021). Erionite-Na from Jersey, with Na^+ as prevailing extra-framework cation, can adsorb the intracellular and cytosol K^+ and Ca^{++} in its micropores

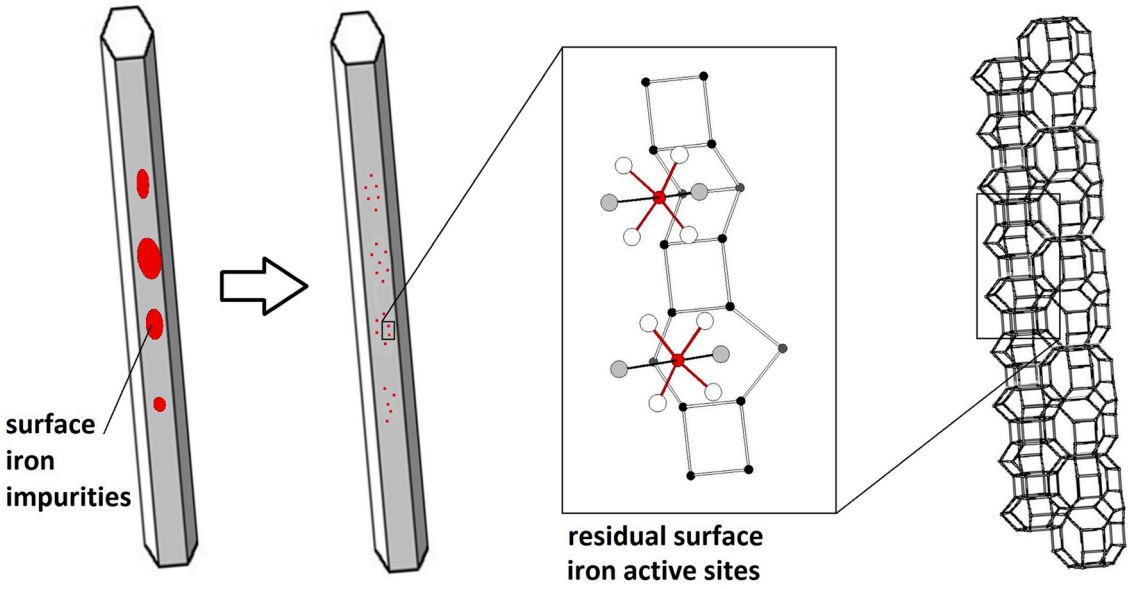

Fig. 10. Iron rich nanophases at the surface of the erionite fibres are not stable in contact with the acidic environment of the phagolysosomes during phagocytosis and may leave a residue of active isolated iron atoms anchored at specific sites like the 6-membered window of the zeolite channels. These isolated active sites can trigger production of hydroxyl radicals.
(modified after Gualtieri et al., 2016).

and release Na^{+}. Moreover, it is known that apoptosis is regulated by endoplasmic reticulum-mitochondria Ca^{2+} cross-talk. Functionally, the stress of the endoplasmic reticulum (ER) triggered by the cell survival process increases the concentration of cytosolic Ca^{2+} via the membrane receptor IP3R-ER promoting the onset of the mitochondria-regulated apoptosis process (Veeresh et al., 2019). If erionite fibres exchange their extra-framework Na^{+} with the cytosolic Ca^{2+}, modifications in the cytosolic Ca^{2+} concentration can alter the ER-mitochondria cross-talk and restrain the mitochondrial apoptotic pathways. Alterations of intracellular calcium homeostasis are responsible for oxidative stress (Donaldson et al., 2007). Zeolite induced modification of the cellular calcium homeostasis has also been reported for other non-fibrous zeolites such as clinoptilolite (Katic et al., 2006) whose cation exchange prompted the Ca^{2+} receptor to activate many phosphatases, kinases and BAD proteins as a result of changed concentration of ions in the cells.

Recently, Ballirano et al. (2015) and Pacella et al. (2017a) showed that erionite can also adsorb iron in acidic environment. In principle, it is possible that iron atoms dissolved from the surface impurities or biological iron are adsorbed in the micropores of the zeolite and Fe^{2+} fixed inside the erionite cage where it is six-fold coordinated to water molecules. In very diluted Fe^{3+} solutions (below 50 mM $FeCl_3$), Pacella et al. (2017b) found that a significant fraction of Fe^{3+} is also adsorbed in the erionite micropores albeit with a significantly lower efficiency with respect to Fe^{2+}. Hence, in iron-loaded zeolites like erionite, the iron sites with very low nuclearity, located in well-defined crystallographic positions, may represent active catalytic sites for successive (chronic) formation of ROS.

From the scenario described above, it is clear that the adverse effects of erionite in vivo can promote an inflammatory activity. Carbone and Yang (2012), working with human mesothelial cells, have shown that erionite fibres induce necrotic cell death with the resultant release of HMGB-1 in the extracellular space. HMGB-1 release causes a chronic inflammatory response, macrophage recruitment and the secretion of the cytokine TNF-α which in turn activates NF-kβ, leading to the survival of mesothelial cells that have accumulated genetic damage due to exposure to mineral fibres.

If we assume the sequence of patho-biological events described earlier for the "asbestos" fibres and that fibre residence in the lung, causing chronic inflammation, is a requisite for the onset of lung cancer (Kamp, 2009), it is plausible that single fibres of erionite, generally shorter than "asbestos" fibres, can be more effectively cleared and translocated to the pleural space. The result is a lower probability to onset lung cancer than a single fibre of crocidolite. As seen for chrysotile, it is obvious that a chronic long-term exposure to erionite fibres (influence of the dose) has the same or even more detrimental effect than a chronic exposure to single crocidolite fibres. The relationship between the physical clearance of short erionite fibres from the lungs and the lower potency for inducing lung cancer with respect to crocidolite is another relevant matter requiring new solid evidences. Literature data seem to support a lower potency of erionite in inducing lung cancer if compared to MM (Hardy and Aust, 1995). Carbone et al. (2011) stated that in Cappadocian villages, hit by MM epidemics, the incidence or lung cancer is not significantly increased compared to other regions in Turkey. This indicates that erionite carcinogenesis is not exactly comparable to "asbestos" carcinogenesis, since "asbestos" causes lung cancer morbidity. Bariş et al. (1996) studied the mortality in three villages in the Cappadocian region of Central Anatolia, exposed to erionite, and reported that between 1970 and 1994, there were 177 (58 %) cancer related deaths in Karain, with 150 (49.2 %) MM cases and only 4 deaths (1.3 %) from lung cancer including two non-smoking females. Between 1980 and 1994, there were 257 cancer related deaths in Tuzköy (where erionite has a mean fibre length of 5.6 μm: Dumortier et al., 2001) and Sarihidir with 120 cases of MM and only 14 patients with lung cancer (4 of whom were non-smoking women). Later, Emri et al. (2002) confirmed that erionite is the main cause of MM in Turkey and did not even mention cases of lung cancer induced by the exposure to erionite. The case of a rural community in Central Mexico (Tierra Blanca de Abajo in the municipality of San Miguel de Allende, Guanajuato), exposed to erionite (with mean length of 6.93 μm) in the natural environment, is apparently in contradiction with the literature data. In fact, Ortega--Guerrero et al. (2015) reported 14 deaths due to different neoplasms of the lungs and only 4 deaths due to MM (Ortega-Guerrero et al., 2015). Saracci (2015) explained such inconsistency with a bias in the estimation of the ratio between lung cancers and mesotheliomas in the whole literature on the subject, due to the smoking habits and diagnostic variability (McCormack et al., 2012). Saracci (2015) remarked that lung cancer was recorded as nearly four times more frequent than MM in Tierra Blanca whereas the opposite held for Karain, where only a few

cases of lung cancer were registered.

Incapsulation inside "asbestos" bodies to halt inflammation chronicity is less probable for erionite because the fibres are generally short and easily engulfed by macrophages. In this regard, Morgan and Holmes (1985) stated that the probability of a fibre becoming coated is determined by its size and increases for fibres longer than 10 μm. In human lungs, there are few coated fibres below this critical length. We have recently seen that in rats exposed via intrapleural inoculation to erionite fibres with a mean length of 3.2 μm, "asbestos" bodies were not found in the tissues even at long residence times (Bursi Gandolfi et al., 2016). Hence, if "asbestos" bodies nucleate only on long fibres, they should not be observed on short erionite fibres that are tentatively engulfed by AM (but not dissolved due of their biodurability). "Asbestos" bodies have been actually observed in BALF of villagers of Tuzköy (Turkey) exposed to erionite with a mean fibre length of 5.6 μm but a significant fraction (35.6 %) longer than 8 μm (Dumortier et al., 2001) and in longer erionite fibres in a case subject from North America (Kliment et al., 2009).

Environmental and epidemiological studies demonstrated that, besides MM, erionite caused lung fibrosis in Cappadocian (Turkey) villages (Emri et al., 2002). Chest radiographs disclosed that 15–39 % of the inhabitants over 25 years of age in the Tuzköy village had diffuse interstitial pulmonary fibrosis (Baris et al., 1988; Dikensoy, 2008). Other literature data clearly indicated that erionite has fibrogenic potential (Suzuki, 1982; Suzuki and Kohyama, 1984) and is responsible for the formation of interstitial fibrosis (Carbone et al., 2011; Kliment et al., 2009). To date, there are no epidemiological data comparing the fibrogenic potential of erionite to that of "asbestos" minerals and few in vitro and animal studies (see for example the work of Suzuki, 1982). Although it is possible to speculate that if an erionite fibre has a lower probability to onset lung cancer than a single fibre of crocidolite, it also has a lower probability to onset lung fibrosis. The potency of erionite vs. "asbestos" in inducing lung fibrosis also requires further experimental evidences.

5.9. *Erionite in the pleural cavity*

Moving to the biological interaction in the pleural space, it was shown earlier that the biodurability of an erionite fibre inhibits its biochemical dissolution during phagocytosis and that the shorter overall size with respect to "asbestos" reduces the probability of incapsulation inside "asbestos" body. On the other hand, translocation of the naked fibre or the AM-engulfed fibre's aggregate to the pleura appears to be a viable mechanism much more effective than that observed for a chrysotile or a crocidolite fibre.

Short erionite fibres are efficiently removed through the lymphatic system but the longer ones that cannot negotiate the aperture of the pleural diaphragmatic stomata, eventually undergo chronic phagocytosis attempts by pleural macrophages and other phagocytic cells active in the pleural space.

All the adverse effects of biopersistent erionite observed in the alveolar environment, including primary ROS production due to release of surface iron and metals by ion exchange ("Trojan horse effect") and indirect secondary ROS production by macrophages, can occur in the pleural cavity and prompt a chronic inflammatory activity. Chronic inflammatory bursts in turn cause damage of DNA of the adjacent mesothelial cells and initiate the sequence (DNA mutations, epigenetic mutations, anomalous mesothelial cell proliferation …) leading to the onset of MM. The high mesothelioma-genicity of erionite is well known (Carbone et al., 2011). Its potency for inducing mesothelioma in rats has been initially observed by Wagner et al. (1985). Carthew et al. (1992) claimed that erionite has 300–800 times more mesothelioma potency than chrysotile and 100–500 times more such potency than crocidolite when given through intrapleural routes. Other studies in animals showed that erionite was 500–800 times more tumorigenic than chrysotile "asbestos" (Coffin et al., 1992) and 200 times more tumorigenic than crocidolite "asbestos" (Hill et al., 1990). The reason why erionite is more mesothelioma-genic than "asbestos" in both rats and humans is still debated.

Besides the chemical-physical properties (surface iron, cation exchange, biodurability), the genetic susceptibility has been invoked to explain the aetiology of MM and especially the high potency of erionite in inducing MM in humans. Although the discussion of genetic factors is out of the aims of this review, an exception is made here because of the interplay between this extrinsic factor and crystal-chemical features in determining erionite-induced MM. According to Carbone et al. (2011), evidence supporting genetic predisposition to MM comes from the Cappadocian region of Turkey where MM caused epidemic unprecedented in history in the villages of Karain, Sarihidir, and Tuzköy as a result of the inhabitants building their homes from erionite-rich pyroclastic rocks. Carbone and Yang and their research group formulated a model that explains erionite-induced MM morbidity in the Cappadocian populations based on the genetic susceptibility to inherit mutations of the cancer suppressor *BAP1* gene (Carbone et al., 2013). Cells with extensive DNA damage (caused by the exposure to erionite) should undergo programmed death (apoptosis) and not grow into malignancies. BAP1 localizes at the ER where it binds, deubiquitylates, and stabilizes type 3 inositol-1,4,5-trisphosphate receptor (IP3R3), modulating Ca^{2+} release (Fig. 11) from the ER into the cytosol and mitochondria to promote apoptosis (Bononi et al., 2017). Reduced levels of BAP1 in the carriers of the mutated $BAP1^{+/-}$ forms are responsible for the reduction both of IP3R3 levels and of Ca^{2+} flux, preventing $BAP1^{+/-}$ cells that accumulate DNA damage from executing apoptosis. A higher fraction of cells exposed to erionite survives genotoxic stress, resulting in a higher rate of cellular transformation (proliferation of anomalous cells) and higher probability to onset carcinogenesis. The high incidence of cancers in $BAP1^{+/-}$ carriers results from the combined reduced nuclear and

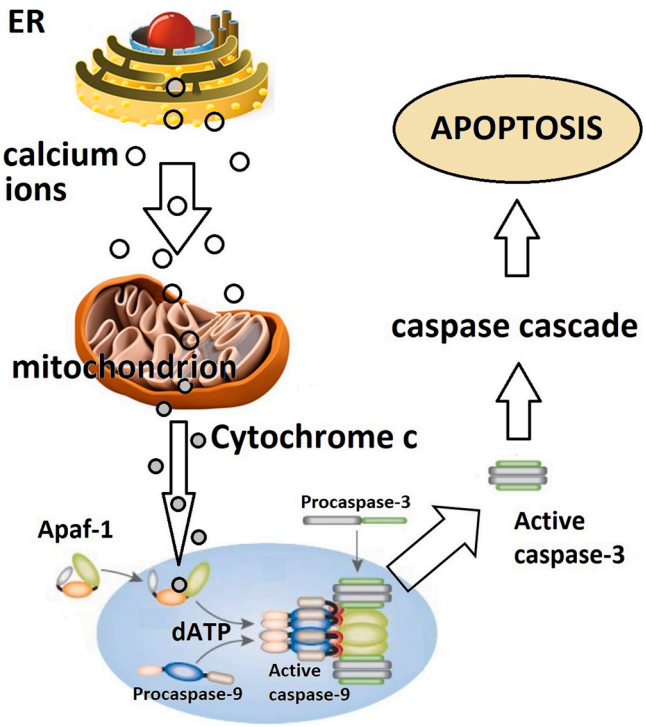

Fig. 11. The action of *BAP1* gene (BAP1 proteins) to promote apoptosis when a cell presents extensive DNA damage (caused for example by the exposure to erionite). BAP1 localizes at the endoplasmic reticulum (ER) where it binds, deubiquitylates, and stabilizes type 3 inositol-1,4,5-trisphosphate receptor (IP3R3), modulating calcium (Ca^{2+}) release from ER in the cytosol to the mitochondrion to promote apoptosis through the caspase cycle (details of the whole process are described in Bononi et al., 2017).

cytoplasmic activities of BAP1 (Bononi et al., 2017).

Hence, according to the model, the families exposed to erionite with inherited carriers of germline *BAP1* gene mutations ($BAP1^{+/-}$) have reduced levels of BAP1 and have a greater probability to develop MM. This would explain why, with the same level of exposure to erionite, some families of the Cappadocian villages are exterminated (carriers of $BAP1^{+/-}$) while others survived (carriers of BAP1). This model should also explain other erionite-induced, "asbestos" induced and sporadic MM cases elsewhere (Nasu et al., 2015) as it was reported mesothelioma clustering in some US families in which up to 50 % of members developed mesothelioma (Testa et al., 2011). In general, somatic BAP1 mutations were found in 20–61 % of two series of MM cases (Yoshikawa et al., 2012; Zauderer et al., 2013).

The model developed by Carbone and Yang is not universally accepted especially if it is generalized to "asbestos" exposure and not only to erionite cases. Betti et al. (2015) showed that five MM families showed germline BAP1 mutations only in one family and concluded that other genes are involved in familial MM predisposition syndrome or that common "asbestos" exposure, which occurred in all of the observed families, is a sufficient cause of MM. Other authors claim that tumour susceptibility or modifier gene(s) other than BAP1 may contribute to the high incidence of mesothelioma and that both "asbestos" exposure and genetic factors play a role in the high rate of mesothelioma and potentially other pleural or lung cancers seen in some families (Cheung et al., 2015). However, no reports exist on the prevalence of germline BAP1 mutations in sporadic MM from population-based case series to confirm the suggestion by Testa et al. (2011) (Betti et al., 2015). In their work, Betti et al. (2015) concluded that the prevalence of germline BAP1 mutations is not a contributing factor to the epidemics of pleural MM that are observed in "asbestos" town of Casale Monferrato (Italy) and BAP1 mutations are probably not a major contributing factor for sporadic MM in general.

The examination of the epidemiological data from the literature evidences the need for further experimental evidence to support the Carbone and Yang model. There is probably a missing piece of information to draw a conclusive comprehensive picture able to explain why BAP1 carriers, exposed to erionite (or "asbestos" minerals), also develop MM and why carriers of ineffective BAP1-mutations, exposed to erionite

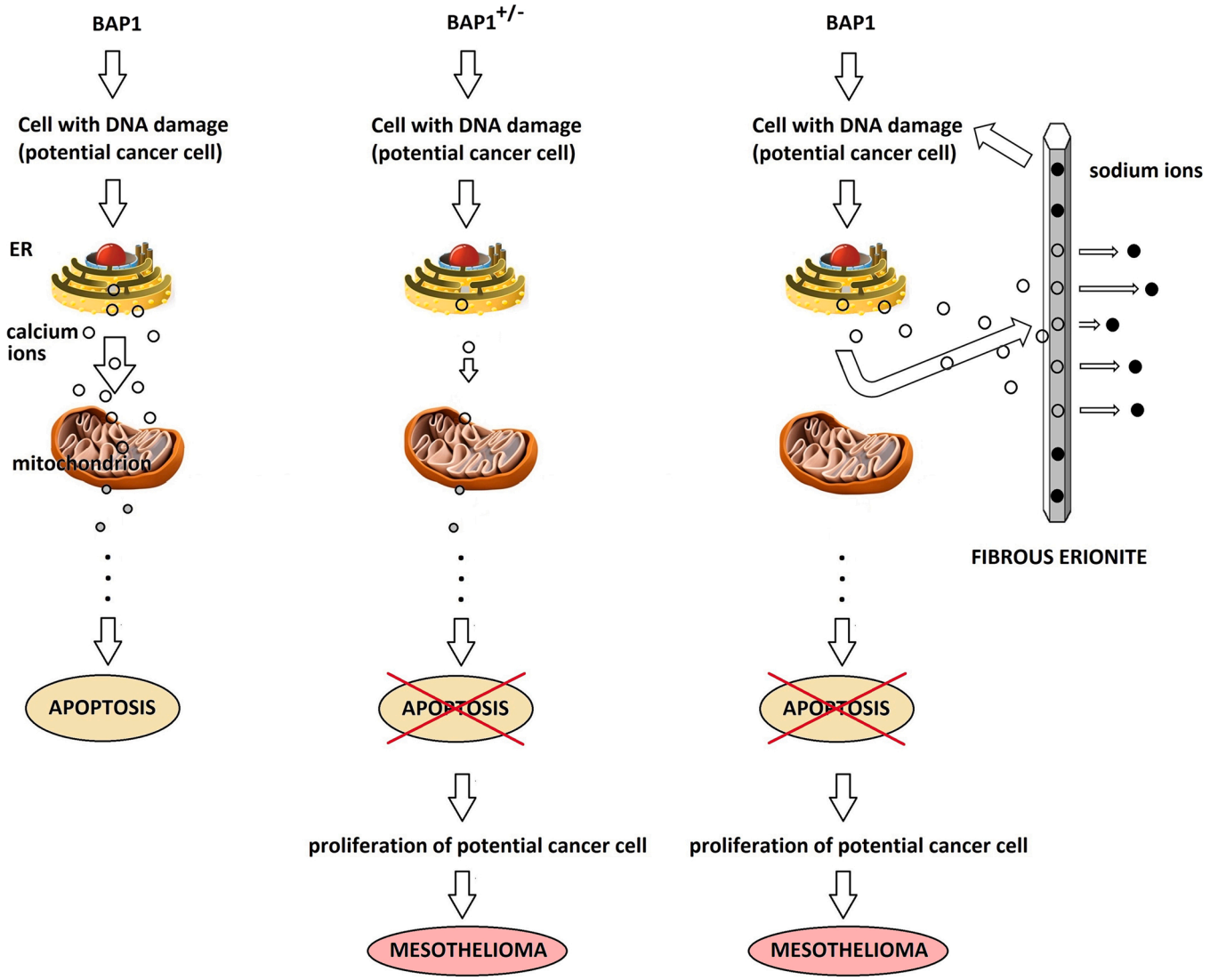

Fig. 12. Mutant forms of BAP1 ($BAP1^{+/-}$) have a reduced stimulation of the endoplasmic reticulum (ER) to bind, deubiquitylate, and stabilize type 3 inositol-1,4,5-trisphosphate receptor (IP3R3) and subsequent very low calcium release from ER in the cytosol to the mitochondrion. Hence, apoptosis through the caspase cycle is not activated and anomalous cells with DNA damage are free to proliferate and onset carcinogenesis. Erionite fibres can cause DNA damage and prompt the action of BAP1 but the calcium cross-talk between ER and the mitochondrion to activate apoptosis is reduced or interrupted because calcium ions are drained inside the micropores of erionite by cation exchange with the sodium ions that are in turn released in the cytosol or extracellularly. Like the case of $BAP1^{+/-}$, apoptosis is not activated and anomalous cells with DNA damage can proliferate and onset carcinogenesis.

(or "asbestos" minerals), do not develop MM. Said that exposure and dose rates play certainly a key role, may the peculiar exchange properties explain erionite excessive potency in inducing MM? Both extracellular and intracellular cation exchange can occur with the sequestration of cations inside the micropores of erionite. The lymphatic system contains a highly proteinaceous (albumin) fluid with prominent electrolytes (Na^+, K^+ and Ca^{2+}) (Skinner et al., 1988) and cation exchange by erionite can influence both the extracellular and intracellular homeostasis (physiological equilibrium). This is valid especially for sodium rich-erionites because it is known that erionite-Na prefers Ca^{2+} ions over Na^+ ions at low levels of divalent ion loadings (Sherry, 1979). Extracellularly, cation adsorption may interfere with the cytosol to extracellular space calcium (Ca^{2+}) ATPase pump through the cell membrane. Intracellularly, erionite can exchange its extra-framework cations with the cytosolic cations (mostly K^+) and modifications in the cytosolic ion concentration can eventually alter the ER-mitochondria cross-talk (calcium ATPase pump) and in turn restrain or interrupt the mitochondrial apoptotic pathways in the same way as the lack of BAP1 protein (substituted by the modified forms $BAP1^{+/-}$) does (Fig. 12). Hence, it is possible that intracellular ion exchange is a co-factor in determining the mesothelioma-genicity of erionite like genetic susceptibility. Research is in progress to verify this model.

Di Giuseppe et al. (2022) observed that erionite fibres engulfed by M0-THP-1 cells prompt release of Na^+ into the cytosol via cation exchange, causing alteration of ion homeostasis and M0-THP-1 cells injury in the early steps of the fibre-cell interation. M0-THP-1 cells activate a compensatory mechanism in which the concentration of cytosolic Na^+ is reduced through osmotic influx of water leading to cell swelling and lysis.

Regarding the potency of erionite in inducing pleural fibrosis, Johnson and Wagner (1989) evaluated the effects up to 12 months on rats exposed to aerosols containing crocidolite or erionite fibres and observed pleural alterations consisted of collagen deposition in the sub-mesothelial connective tissue layer and fibrosis in erionite-exposed rats. It is interesting to consider the work of Suzuki and Kohyama (1984) who discovered that peritoneal fibrosis was the most common disease responsible for death of mice groups intraperitoneally inoculated with fibres of different nature. These authors reported fibrosis-related incidence of death or serious sickness in animals exposed to high doses of amosite (39–74 %), UICC chrysotile (42 %) and erionite (89 % for erionite II in that paper). If we refer to these results and assume comparable effects for the peritoneum and pleura, it is possible to conjecture that an indigestible erionite fibre has a fibrogenic potential at least comparable to that of crocidolite, one of the most fibrogenic mineral fibre (Shukla et al., 2003), and greater than that of digestible chrysotile. This issue adds to the long list of points of discussion that require conclusive experimental evidences.

6. Concluding remarks

Patho-biological effects of mineral fibres leading to adverse effects in vivo have been revised under the mineralogical perspective. Mineral fibres with different crystal-chemical assemblages (amphiboles, layer silicates and zeolites) behave differently both in vitro and in vivo and display different toxicity/pathogenicity potential.

The description of the patho-biological paths of mineral fibres revealed that many issues are still open and call for further studies. Below is a summary of the most relevant facts, assumptions and open issues discussed in this review and made by considering a one-to-one comparison between the different fibre species.

A crocidolite fibre has a higher probability than chrysotile and erionite fibres to reach the deep respiratory tract. The comparison between chrysotile and erionite fibres is more difficult because on one hand acicular erionite fibres are more prone to bypass the upper bronchial/bronchiolar tracts than curled chrysotile fibres. On the other hand, erionite lower density does not favour its descent down to the alveolar space.

Dissolution by alveolar macrophages (AM) is more efficient for chrysotile with respect to crocidolite fibres because chrysotile is not biodurable. Hence, chrysotile in principle should be less toxic and pathogenic in the lungs than crocidolite. Actually, recent in vitro findings suggest that a 'Trojan horse effect' may occur when chrysotile dissolves making the metals hosted in its crystal structure bioavailable in the lungs. Moreover, very high exposure animal studies suggest that corroded chrysotile fibres leave behind a silica-rich amorphous structure that continues to induce frustrated phagocytosis and cell disturbance. Because of the release of metals during dissolution, ROS production by chrysotile on a short-medium term is observed while indigestible crocidolite fibres prompt less intense but chronic ROS production. It is unknown to date whether the probability of DNA damage of alveolar cells leading to carcinogenesis is higher for intense acute events prompted by chrysotile than for crocidolite-induced mild but chronic events.

The peculiar chemical-physical nature of the erionite fibres (surface iron, release of toxic metals by cation exchange, cation exchange of extracellular and intracellular ions) results in differences in the behaviour of this mineral during the phagocytosis process with respect to "asbestos" fibres.

Assuming the sequence of patho-biological events leading to lung cancer, and under the proviso that chronic inflammation is a requisite for its onset, it is reasonable to assume that a single fibre of chrysotile has a lower probability to initiate lung cancer than a single fibre of crocidolite.

Although the mechanism of fibres' translocation from the alveoli to the pleural cavity is still unclear, it is possible to speculate that single fibres of erionite, generally shorter than "asbestos" fibres, can be more effectively translocated to the pleura reflecting in a lower probability of an erionite fibre to onset lung cancer than a crocidolite fibre. This model that explains the lower potency for inducing lung cancer with respect to crocidolite needs further experimental evidence.

Another point of discussion is whether fibrosis is more likely activated by intense acute events as those prompted by chrysotile or by less intense chronic events as those produced by crocidolite activity.

Long indigestible crocidolite fibres can be incapsulated inside "asbestos" bodies. To date, it is still unclear whether iron-rich fibres contribute to the nucleation and growth of "asbestos" bodies. Notwithstanding, based on high dose in vitro studies, the contribution of inert biodurable crocidolite should be negligible while non biodurable chrysotile may in principle act as active pool for the growth of iron hydroxides forming the coating. Although this matter is under discussion, there is agreement in the literature that the contribution to the "asbestos" bodies of inorganic iron from the fibres is minor to null.

Incapsulation inside "asbestos" bodies should be less probable for erionite because the fibres are generally short and easily engulfed by macrophages.

The biodurability of an erionite fibre inhibits its biochemical dissolution during phagocytosis and the shorter overall size with respect to "asbestos" reduces the probability of incapsulation inside "asbestos" body. On the other hand, translocation of the naked fibre or the AM-engulfed fibre's aggregate to the pleura appears to be a viable mechanism much more effective than that observed for a chrysotile or a crocidolite fibre.

Crocidolite fibres can easily accumulate in the pleura while chrysotile not. Because of their low biodurability and curled shape, chrysotile fibres are more difficult to translocate to the pleural space than crocidolite fibres. If we assume that a chrysotile fibre is unlikely to reach the pleura, its potency for inducing MM should also be reduced with respect to amphibole fibres. The supposedly lower potency of chrysotile in inducing MM obviously does not mean that chrysotile itself cannot induce MM.

The high potency of fibrous erionite of inducing mesothelioma is universally accepted but the causes why erionite is apparently more

mesothelioma-genic than "asbestos" are still debated. Besides the chemical-physical properties, the genetic susceptibility has been invoked to explain the aetiology of MM. The inspection of the epidemiological data from the literature evidences the need for further experimental data to support the model of genetic predisposition. Other co-factors, like the cation exchange inside the erionite micropores, may contribute in determining the overall mesothelioma-genicity of erionite but research is in progress to verify this additional model.

Although the formation in situ of "asbestos" bodies in the pleural space is not demonstrated yet, the formation of coated long biodurable crocidolite fibres in the pleural cavity should be possible in principle and more likely than that of "asbestos" bodies coating non-biodurable chrysotile fibres and shorter biodurable erionite. This is another poorly explored topic to date.

Crocidolite fibres are among the most potent mineral fibres for inducing fibrosis in the lungs and are expected to be even more fibrogenic in the pleura. Oppositely, if a chrysotile fibre is unlikely to reach the pleura and its potency for inducing MM is subordinate to that of a biodurable amphibole fibre, its potency for inducing pleural fibrosis should also be lower. The supposed lower potency of chrysotile for inducing pleural fibrosis is another point to be further investigated.

Regarding erionite, there are no epidemiological data to date comparing the fibrogenic potential of erionite to that of "asbestos" minerals and few in vitro and animal studies. Nevertheless, it can be speculated that an indigestible erionite fibre has a fibrogenic potential at least comparable to that of crocidolite and greater than that of digestible chrysotile.

Environmental implication

This review presents the state of the art on the bio-chemical mechanisms leading to lung carcinogenesis induced in vivo. The mineral fibres described here (the asbestos minerals crocidolite and chrysotile, and the zeolite erionite) are well-known airborne hazardous materials classified by the International Agency for Research on Cancer (IARC) as "carcinogenic to humans (Group 1)" and causing lung cancer and mesothelioma in humans. The review helps addressing environmental problems because it explains the toxicity and pathogenicity mechanisms of chrysotile, still used worldwide in a "safe mode", vs. amphibole asbestos and erionite, contributing to the solution of the "global chrysotile issue".

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Alessandro F. Gualtieri reports article publishing charges and travel were provided by University of Modena and Reggio Emilia.

Data Availability

Data will be made available on request.

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Disclaimer

The opinions expressed in this review are those of the author only and may not reflect the opinions or views of members of the "asbestos" scientific community.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhazmat.2022.130077.

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