# Fluoro-edenite and carbon nanotubes: The health impact of 'asbestos-like' fibres (Review)

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**Abstract.** Several decades have passed since Wagner *et al* demonstrated a causal link between asbestos fibre inhalation and the development of pleural mesothelioma in 1960. It was later suggested that pleural plaques are a benign consequence of exposure to these fibres. Most recently, a significant association between exposure to asbestos and cancer diagnosed at various sites, such as the peritoneum, stomach, pharynx, colon and ovaries has been demonstrated. The great concerns about public health that arose from the scientific evidence presented above have led to the banning of asbestos in several countries. Over the years, the suspicion that particles with a high aspect ratio may have asbestos-like pathogenicity has been supported by increasing evidence. Natural occurring minerals, as well as man-made fibres, have proven capable of inducing either chronic inflammation of serous membranes, or, in some cases, the development of peritoneal and pleural mesothelioma. The pathogenic role of both fluoro-edenite and carbon nanotubes, two 'asbestos-like' fibres is summarized and discussed in this review. The data presented herein support the notion that occupational exposure to these two types of fibre contributes to the development of different types of cancer.

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## 1. Introduction

Several studies have been performed on the pathogenicity of asbestos and fibres in general (1), and the results have demonstrated that different types of fibre (vitreous, ceramic and organic) can produce similar pathological effects.

This evidence has led to the conclusion that the pathogenetic mechanisms of action of these fibres may be related not only to their chemical properties, but also and more significantly to something they share: the physical structure.

In 1972, Stanton and Wrench compared different fibres (asbestos, aluminum oxide and fibrous glass) to find a common link to their pathogenicity which seemed to be their peculiar, needle-like shape (2). During the following years, a pathogenicity paradigm was elaborated: this paradigm takes into account the physical characteristics and not the chemical structure of a compound (except when the chemical structure influences biopersistence) to evaluate its pathogenicity and carcinogenic potential (3,4). In the present review, the pathogenic role of two 'asbestos-like' fibres, fluoro-edenite and carbon nanotubes, is summarized and discussed.

## 2. Physical structure

Diameter and length are the most important determinants of the toxic potential of a particle, and in particular, the aerodynamic diameter (Dae) is the key measure which can be used to predict how far a particle can travel into the respiratory system (5).

The lower respiratory tract, in fact, is endowed with several clearance mechanisms that allow the removal of small particles (6). The most relevant of these mechanisms is the ciliated epithelium, which is present from the trachea to the main bronchi. Besides, the activity of the alveolar macrophages grants the removal, via phagocytosis, of particles that are able to reach the most distal part of the lower respiratory tract. Nonetheless, sometimes macrophages are not able to phagocytize and dissolve some of these foreign bodies, resulting in what is known as 'frustrated phagocitosis' (7) and this subsequently leads to chronic inflammation (8) and in some cases, to the development of cancer.

Fibres with a diameter of ≤1 $\mu$m can be inhaled and may surpass the ciliated epithelium. Length, generally, does not have a signficant impact on the aerodynamic diameter, but it can play a significant role if the fibre is long enough to impact on the airways walls, resulting in deposition.

Nevertheless, length is considered a key factor in fibre pathogenicity, and this hypothesis is strongly supported by several authors (9,10). Longer fibres, in fact, are hardly enclosed into macrophages, impairing phagocytosis and thus persisting into the pulmonary interstitium (11).

According to this statement, Stanton *et al* intestigated the damage caused by fibres of different lengths on the pleural mesothelium, concluding that fibres of >8 $\mu$m in length pose a higher pathogenic hazard (5).

Similar results were also obtained by other authors (12,13), who demonstrated a major risk for long asbestos fibres vs. short asbestos fibres in experimental studies and with different types of asbestos (amphiboles and serpentines) (14).

## 3. Biopersistence

It is common knowledge that the deposition of volatile particles in the lungs can lead to inflammation (12,15); and also to the development of numerous pathologies, such as fibrosis (16), pleural plaques (17), pneumoconiosis (18) and cancer (19). However, even if a particle can cross the ciliated epithelium and reach the distal part of the respiratory apparatus, it does not mean that it will be able to accumulate.

As previously demonstrated, the clearance of small particles in the lowest parts of the respiratory system is performed by the alveolar macrophages (20). Macrophages phagocytize foreign bodies and then they can either dissolve them in an acidic milieu by the action of several enzymes (catalases and peroxidases), or migrate towards the ciliated epithelium.

Some particles tend to spontaneously break or split longitudinally, consequenlty allowing for phagocytosis to be carried out more easily by the immune system, while other particles seem to be much more resilient (21).

Biopersistence refers to the ability or capacity of a molecule to maintain its chemical and physical characteristics in an organic ambient, the pulmonary milieu in this case. In other words, the more a compound structure can resist the catalytic processes listed above, the more this molecule will be biopersistent and may thus determine the pathological effects (22).

However, in some cases, even a poorly biopersistent molecule can cause inflammation and toxicity. If the breakdown of a particle leads to the release of toxic or reactive molecules, this may then lead to pathological consequences. The deposition of heavy or toxic metals is one of the most common events in this specific case.

## 4. Data collection methods

In the present review, a computerized search on PubMed was performed up to September 2015. The search terms used were 'asbestos-like fibres', 'fluoro-edenite', 'carbon nanotubes', 'fibres' and 'mesothelioma'. Attention was paid to fibres which were presented in the highest number of experimental studies. Only original studies, either *in vivo* or *in vitro*, were included, and a chronicle of the earliest most important studies to the latest findings was made (Table I). Due to the lack of human epidemiological studies in some cases, studies using animal models were given precedence over *in vitro* studies.

## 5. Fluoro-edenite

Fluoro-edenite is a mineral fibre of volcanic origin, which is closely related to edenite, a much widely represented mineral.

The substitution of a hydroxyl group (-OH) with a fluoride (F) is the only difference in the chemical structure between these two amphiboles (edenite and fluoro-edenite) (23,24); however, this small difference means that fluoro-edenite has a very different impact on human health.

Identified in 1997 near the city of Biancavilla (Sicily, Italy), on the slopes of the Etna volcano, this mineral was extracted from the quarries in Monte Calvario, and was widely used as a building material for road paving and buildings. Very similar amphiboles have also been found in Japan; however, no significant impact on public health was observed in this country, possibly since the mineral was neither mined nor used in the building industry.

The scientific community began to investigate fluoro-edenite when an incidence of pleuric mesothelioma was registered in the area of Biancavilla by an epidemiological survey, conducted from 1988 to 1997 (25).

Pleural mesothelioma is, in fact, a fairly rare malignant tumour, which has been linked very strongly with the occupational exposure to asbestos fibres, and it rarely occurs in indiviuals not exposed to these fibres. Furthermore, since pleural mesothelioma is closely linked to exposure to asbestos fibres, male mine workers are more frequently affected than woman.

Oddly, the epidemiological pattern of pleural mesotheliomas in Biancavilla differed from the 'classic' professional exposure-related one, since this type of cancer equally affected males and females, without any correlation to exposure to asbestos fibres, and also affected young adults. This type of pattern is more likely related to an environmental exposure (25,26).

Dust rising from the unpaved roads, and due to building construction and demolition, as well as the mining activities in Monte Calvario, were in fact all responsible for the inhalation of fluoro-edenite fibres by the resident population.

While fluoro-edenite was considered the agent most likely responsible for the development of pleural mesotheliomas, experimental studies were still needed to support that hypothesis. Soffritti *et al* noted a marked increase in cases

Table I. Experimental studies discussed in this review.

| Author/(Refs.) | Year | Fibre | Type | Object of the study |
|---|---|---|---|---|
| Cardile *et al* (31) | 2004 | Fluoro-edenite | *In vitro* | Citotoxicity comparison between fluoro-edenite and crocidolite asbestos amphibole |
| Cardile *et al* (32) | 2007 | Fluoro-edenite | *In vitro* | Evaluation of Hps70 induction by 2 different forms of fluro-edenite, and tremolite asbestos |
| Musumeci *et al* (35) | 2011 | Fluoro-edenite | *In vitro* | Assessment of Rb/pRb role in fibre-mediated cancer development |
| Poland *et al* (41) | 2008 | Carbon nanotubes | *In vivo* | Effects of CNT exposure on rodent's peritoneal surface |
| Takagi *et al* (42) | 2008 | Carbon nanotubes | *In vivo* | Comparison between MWCNT, fullerene and crocidolite asbestos in peritoneal cancer development after perironeal injection in $p53^{+/-}$ mice |
| Muller *et al* (45) | 2009 | Carbon nanotubes | *In vivo* | Comparison between MWCNT+ and MWCNT- in peritoneal cancer development after peritoneal injection, over a period of 24 months |
| Schinwald *et al* (46) | 2012 | Carbon nanotubes | *In vivo* | Broad spectrum comparison between different types of fibres to assess a threshold length for deposition |
| Albini *et al* (47) | 2015 | Carbon nanotubes | *In vivo* | Simulation of occupational exposure to CNT in a novel murine model |

Rb, retinoblastoma; CNT, carbon nanotube; MWCNT, multi-walled carbon nanotubes.

of mesotheliomas in rats injected with fluoro-edenite fibres into their peritoneal cavities (27). In addition, Ballan *et al* performed an *in vitro* study comparing the effects of prismatic fluoro-edemite and fibrous fluoro-edenite on A549 cells (a tumour cell line derived from human lung carcinoma with the properties of alveolar epithelial cells). While the prismatic form of fluoro-edenite did not exhibit significant carcinogenic activity, the fibrous form induced marked changes in cell morphology, promoting multinucleation, a significant decrease in the number of viable cells, and, moreover, these changes did not induce apoptosis in the cultured cells (28).

These findings, even if not conclusive, suggested great caution in the management of fluoro-edenite and, together with the local institutions, an environmental health action plan was implemented. Many efforts were spent in the reclamation of the area by repaving the roads with safe materials, closing the quarries, sealing them with bitumen spray and safely removing the building industry byproducts contaminated with the amphibole.

Since 2000, various environmental surveys were carried out with the aim to determine the concentrations of the amphiboles in the area of Biancavilla, and, over the years, to prove the effectiveness of the reclamations. A consistent decrease in the environmental levels of the amphibole was registered over time in the outdoor areas of the town, although the real level of indoor exposure to fluoro-edenite has yet to be determined (29).

Finally, the International Agency for Research on Cancer (IARC) has recently classified fluoro-edenite fibrous amphibole as carcinogenic to humans (group 1) (30). Even after all these studies were performed and all the data presented, much has yet to be learned from the case of Biancavilla. Further studies are required in order to determine the occupational activities that may be responsible for the increased exposure to fluoro-edenite. Moreover, it is very difficult to estimate the levels of exposure of the local population inside their homes. Epidemiological surveys on the resident population have to be carried out, in order to detect the early signs of mesothelioma, pneumoconiosis and other respiratory diseases that may be related with the inhalation of amphiboles. Furthermore, the individuation of a biological marker would be of great value to the epidemiological surveillance.

**6. Pathogenicity of fluoro-edenite**

Beginning from the early 2000s, Cardile *et al* performed several studies on the possible pathogenic or even carcinogenic effects of fluoro-edenite. Their studies aimed to discover the cause of the excessive mortality caused by mesothelioma that was registered in the area of Biancavilla, as has already been mentioned above.

In 2004 in one of their first studies (31), the authors tried to evaluate the carcinogenic potential of fluoro-edenite by comparison with crocidolite, a very well-known and toxic asbestos amphibole. In their study, different types of cells (human lung fibroblasts, the human lung alveolar epithelial cancer cell line A549, and the mouse monocyte-macrophage cell line J774) were exposed to 5/50/100 $\mu$g/ml of fluoro-edenite or 5/50/100 $\mu$g/ml of crocidolite, and compared to an untreated control.

Fluoro-edenite, as well as crocidolite (even if the latter exhibited greater toxicity) induced DNA damage, a decrease in cell metabolism, an increase in the release of lactate dehydrogenase (LDH), a concentration and time-dependent increase in reactive oxygen species (ROS) production, and an overall decrease in cell viability. Indeed, both minerals induced oxidative stress in the cultured cells but through different mechanisms: crocidolite contains iron and can lead to ROS formation directly; fluoro-edenite, on the other hand, seems to interfere with the mitochondrial respiratory chain, causing oxidative stress indirectly. This evidence led to the conclusion that fluoro-edenite is possibly carcinogenic to humans.

In another study published in 2007 by the same authors (32), mesothelial cells were cultured with three mineral fibres: tremolite, and two forms of fluoro-edenite with different iron contents. The aim of their study was to further evaluate the *in vitro* effects of these two fibres, and to shed light on the Hsp70 response following exposure to these amphiboles. The results revealed an increase in free radicals, and a consequent increase in stress-induced proteins, such as Hsp70, in an iron content-dependent manner. Hsp70 is one of the most widely expressed stress-induced proteins in the lungs, and its function is to protect cells from acute injury (i.e., the one caused by fibres) (33). This heat shock protein though, has been proven to be highly immunogenic (34). A persistent injury caused by the inhalation of highly biopersistent materials, such as fluoro-edenite can lead to steadily increasing levels of Hps70, which at first may have a cytoprotective effect, but at later stages, can trigger autoimmunity, thus aggravating inflammation.

Musumeci *et al* (35) performed a study in which human bronchoalveolar alveolar epithelial cells (A549) and human non-malignant mesothelial cells (MeT-5A) were incubated with increasing concentrations (10, 50 and 100 $\mu$g/ml) of fluoro-edenite to estimate the expression of retinoblastoma (Rb) protein, the dysfunction of which is known to be involved in cancer development. The results revealed a dose-dependent increase in the phospho-retinoblastoma (pRb) levels in the cultured cells. Oddly, the Rb levels were not significantly altered. Furthermore, the pRb/total Rb relative ratio always exceeded 50% in the sample which showed pRb overexpression. The expression of cyclin D1 and its inhibitor p27 was also evaluated, and the cells incubated with 50 and 100 $\mu$g/ml of fluoro-edenite exhibited a significant increase in cyclin D1 levels, whereas the p27 protein level was decreased. These effects were observed in both the A549 and MeT-5A cell lines. The key role of pRb in lung cancer development has already been demonstrated in a previous study (36), and the results of that study led to the conclusion that the functional failure of pRb may be a critical factor in fibre-induced cancer development.

## 7. Carbon nanotubes

A carbon nanotube (CNT) is an allotrope of carbon with a cylindrical nanostructure. These molecules, formed exclusively by carbon atoms, are present in the form of single-walled carbon nanotubes (SWCNT), which are formed by a single graphenic layer and measure 1-3 nm in diameter, or multi-walled carbon nanotubes (MWCNT), that are multiple graphenic layers folded together, coaxially, with a total diameter of 10-200 nm. The length of these particles can vary from less than a micrometer to several micrometers.

Carbon nanotubes were described for the first time in 1952 by Radushkevich and Lukyanovich (37). Since the article was only published in Russian during the time of the cold war, when scientific communications between Western countries and Soviet ones were limited, carbon nanotubes only received global attention in the late 1990s, when Iijima published an article in 'Nature' describing this discovery (38). In the following years, carbon nanotubes received great interest due to their physical properties: a single-walled nanotube, in fact, is an extremely traction-resistant material and a flexible one as well. Moreover, chemical modifications to the molecular structure of SWCNTs can alter their electrical conduction capabilities, making them either conductors or semi-conductors (39). These are only few of the many characteristics of carbon nanotubes, but it is sufficient to explain the great diffusion of these materials, from the electronic industry to the biochemical applications.

However, the thin, low aspect ratio shape of carbon nanotubes is dangerously similar to some naturally occurring fibres, such as asbestos, and great concerns had been raised about the potential toxicity of these materials (40). In particular, workers involved in the production and management of carbon nanotubes may be placing their health at risk.

Since the already said great potential of these new particles in terms of their possible uses and technological development, a number of studies have been performed examining their toxicity.

Studies conducted *in vitro* or on rodents, have demonstrated that carbon nanotubes induce inflammation, fibrosis and finally, mesothelioma in the serosal cavities of rodents (8,15,16,40). At this time, detailed data on occupational and/or consumer exposure to these particles are lacking, as well as data indicating a direct correlation between carbon nanotubes and cancer in humans.

Nevertheless, the IARC working group assessed MWCNT-7 as a possible carcinogen to humans (group 2B); the other forms of MWCNT, as well as SWCNT were assessed as not classifiable as to their carcinogenicity to humans (30). Additional studies are warranted in order to fully determine the real threat that these new, exceptional materials, can pose to the health of both industrial workers and consumers.

## 8. Pathogenicity of carbon nanotubes

In 2008, a number of researchers published their results of the injection of carbon nanotubes into the peritoneal cavity of rodents. Poland *et al* injected a dose of 50 $\mu$g per rodent of long carbon nanotubes, and used short carbon nanotubes, carbon black, long and short fibres of amosite asbestos as the controls. The results revealed the retention of the long fibres at the mesothelial surface of the mouse peritoneum, and marked inflammation and macrophage infiltration with the formation of foreign-body giant-cell granulomas (41).

Another study published in the same year by Takagi *et al* (42) examined the toxicity of MWCNTs in comparison with crocidolite and fullerene. Nineteen $p53^{+/-}$ mice were administered (3 mg/animal) MWCNT via a single intraperitoneal injection, another 19 mice were administered (3 mg/animal) fullerene, and another 19 mice were administered an identical dose of crocidolite. The vehicle solution was administered at the dose of 1 mg, via intraperitoneal injection, to 19 mice as the negative control. The mice in the MWCNT group were the first to reach 100% mortality, and, after the study was completed, an autopsy revealed fibrous peritoneal thickening and a high incidence of macroscopic peritoneal tumours in the mice in the MWCNT group. Similar effects were observed in the mice in the crocidolite group, although to a lesser extent, while the mice in the fullerene group exhibited no pathological signs, apart from black plaques on the serosal surface. $p53^{+/-}$ mice have also been used as a model for asbestos-induced carcinogenesis (43) and, furthermore, this model has proven to be particularly sensitive to ROS-induced carcinogenesis (44) as may be the case with MWCNT. The marked induction of mesothelioma by MWCNT registered in that study (42), compared to the absence of pathological signs produced by the same dose of fullerene (a compound very similar in chemical composition, but lacking the 'asbestiform' needle shape), highlights once again the importance of physical structure and biopersistence in tumourigenesis. Even if very relevant, it is important to contextualize these results. Real life exposure to CNTs is very unlikely to deliver such a dose of long MWCNT to the serosal surfaces of the body, and further studies are warranted to assess the real risks that these particles can pose to human health.

In 2009, an unexpected result, in apparent contrast with the study of Takagi *et al* (42), was registered by Muller *et al* (45). In this study, the authors hypothesized that MWCNT with structural defects, termed MWCNT+, would induce tumour formation, whereas multi-walled carbon nanotubes without structural defects (MWCNT-), would not. To prove this hypothesis, they intraperitoneally injected 3 groups of 50 male Wistar rats with a single dose of MWCNT+ (2 or 20 mg/animal), and 20 mg/animal of MWCNT-. Two groups of 26 rodents were used, respectively, as the negative controls and positive controls, administering 2 mg crocidolite/animal to the latter. After 24 months the experiment was terminated, and the rats were sacrificed. In contrast to what the authors expected, neither the rats administered MWCNT+ nor the rats administered MWCNT- rats exhibited a significant development of mesotheliomas, and the only group with and an above average incidence of this tumour (36%) was the crocidolite-injected one. The authors finally concluded that the most likely reason behind these findings was that the mix of MWCNT administered to rats did not contain a sufficient number of long MWCNT, which are supposed to be crucial for cancer development. The MWCNT+ (20 mg)-injected rats, in fact, exhibited a similar pattern of initial peritoneal inflammation with the crocidolite-injected (2 mg) rats. Only in the latter group, though, cancer ensued inflammation. The peritoneal cavity, similar to the pleural cavity, is capable of clearing short fibres or tangles at a low dose through macrophage action and the stomata, a peculiar anatomical structure of serosal surfaces (4).

A study published in 2012 (46) performed a wide-range comparison between fibres different in length and nature, with the aim to finally discover a threshold length beyond which fibres can have adverse effects on the pleura. Silver nanowires of different mean lengths (3-5-10-14-28 $\mu$m), long amosite asbestos fibres (LFA; 100% >5 $\mu$m) and short amosite asbestos fibres (SFA; 3.1% >5 $\mu$m), short (4 $\mu$m) and long (20 $\mu$m) nickel nanowires, and short (2 $\mu$m) and long (13 and 36 $\mu$m) MWCNT. Ag-nanoparticles, Ni-nanoparticles and carbon black nanoparticles were used as controls. Each one of these materials was injected in the pleura of different mice, that were euthanized after 24 h and 1 week. The results of the examination with electron microscopy and backscatter electron microscopy revealed a length-dependent inflammatory response to silver nanowires >5 $\mu$m and to LFA. By contrast, shorter Ag-NW and SFA did not exert significant inflammation. Interestingly, fibres >5 $\mu$m in length did not determine a further increase in inflammation, eliciting an 'all or nothing' behaviour. According to that study, the clearance of long particles in the pleura through the stomata and the monocyte-macrophage system seems to be limited to particles <5 $\mu$m in length. This study highlighted once more the importance of length in fibre pathogenicity: shorter fibres, in fact, did not trigger an inflammation process as they were not retained on the parietal pleura surface, while longer fibres which could not be cleared via the stomata, caused inflammation.

Based on these findings, it can be speculated that length may be used as a key parameter in the future to discriminate between carbon nanotubes which are 'safe' for human exposure and those that are not, particularly in occupational settings.

In 2015, Albini *et al* produced a murine model with the aim to simulate workplace exposure to CNTs, MWCNTs in particular (47). MWCNTs had a mean diameter of 9.5 nm, a mean length of 1.5 $\mu$m and a surface area of 250-300 $m^2$/g. In this model, mice were exposed to CNTs constantly, as it was mixed in the wood shaving of their litters (1.5 g MWCNT/80 g litter). Other mice were housed under similar conditions, but without the addition of nanotubes, and used as controls with particular care being taken by the authors to avoid contamination between the two groups. The rodents were sacrificed after 1, 2, 3, 4 and 5 weeks to excise internal organs, such as the liver, brain, kidneys, intestine and lungs to evaluate the content of MWCNTs. Even if no overt pathological sign was observed, the results revealed the rapid tissue distribution of MWCNTs. Not only were the lungs noted as a site of deposition, but the liver and the brain were also found to be deposition sites, which were reached through the bloodstream. The concentration in these organs increased in a time-dependent manner. It is known for a fact that CNTs are associated with tissue inflammation (40,48,49) and, according to the literature, the authors researched and found an increase in the number of CD68 (a macrophage marker)-positive cells in animals exposed to MWCNTs for 5 weeks, particularly in correspondence to the sites of major nanotube deposition. The finding of amyloid substance in the sites of major CNT deposition, folded around nanotubes fibrils, once again confirmed what previous studies have already demonstrated, namely that amyloid deposition is a possible consequence to a chronic inflammatory microenvironment (50).

The observed pharmacokinetic patterns indicate inhalation as the main absorption route, even if it was not the only one. This study (47) produced an interesting model of 'real' occupational exposure, and highlighted at least two important factors: MWCNT, via inhalation and via the bloodstream, can rapidly be deposited in many organs and can generate amyloid tissue aggregation in a short period of time; hence, MWCNT-induced amyloid deposition may be a potential risk factor for the development of neurodegenerative diseases, hepatic cirrhosis and several other amyloid-related syndromes, particularly in predisposed individuals.

Further studies are required in order to evaluate the possible health risks caused by these nanomaterials. The last study mentioned (47) indicates that, in the long term, the spectrum of pathologies induced by CNTs may be more than a simple 'asbestos-like' pattern, raising once again great caution about human exposure to these materials.

## 9. Discussion

Fluoro-edenite and carbon nanotubes, although very different in nature, diffusion and utilization, share a similar toxic behaviour towards the lungs and, more particularly, the pleura.

Over the past years, fluoro-edenite has been assessed as carcinogenic to humans in its amphibole form, and many studies have investigated the mechanisms of toxicity of this fibre (27,31,32,51). The area of Biancavilla, where the issue regarding fluoro-edenite began, at this time has been greatly reclaimed, and exposure levels are now certainly lower for the population. Still, there are many questions to be answered. It is impossible to assess the real level of exposure for the inhabitants, and probably certain classes of workers, such as quarry workers, bricklayers and garbage collectors may have been more exposed to dust particles due to their occupation. Furthermore, the long-term effects of this amphibole on humans remain unknown, and a close surveillance has to be carried out. Another interesting question could be about the most appropriate way to screen hundreds of individuals, taking into account the possible differences in past and current exposure levels.

While fluoro-edenite has a very limited importance in terms of diffusion on earth, carbon nanotubes, on the other hand, have raised a much greater interest in the scientific community due to their progressive diffusion in different fields of industrial activities. These man-made, organic materials can exist in different forms, such as tangles, fibres, single-walled and multi-walled nanotubes, with marked differences in their aspect ratio. It has been shown that some forms of CNTs can match asbestos as regards their toxic potential (52), and other studies have clarified the threshold length beyond which deposition and pleural chronic inflammation can occur (46).

Since the production of these materials often generates a mix of different CNTs, it is challenging to determine whether or not the health of workers involved in CNT production may be at risk. This issue is even more complex if we take into account that there is no real data available on the carcinogenicity of these fibres in humans, but, by contrast, there is numerous evidence on the carcinogenicity of MWCNTs in rodents. The latest findings indicate that CNTs can spread throughout the body, and promote amyloid substance apposition, revealing other unexpected scenarios about their toxicity towards humans.

According to the evidence provided, further studies are necessary to assess the risk for humans at real doses and times of exposure and, in the meantime, particular attention has to be given to the protection of the health of exposed workers, as well as that of consumers.

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