The MAK Collection for Occupational Health and Safety

Fibrous Dusts, inorganic

MAK Value Documentation, addendum – Translation of the German version from 2018

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Keywords: fibrous dusts, inorganic; mechanism of action; toxicity; carcinogenicity; genotoxicity; mesothelioma; inflammation; lung; clearance

Citation Note: Hartwig A, MAK Commission. Fibrous Dusts, inorganic. MAK Value Documentation, addendum – Translation of the German version from 2018. MAK Collect Occup Health Saf [Original edition. Weinheim: Wiley-VCH; 2019 Oct;4(4):2054-2107]. Corrected republication without content-related editing. Düsseldorf: German Medical Science; 2025. https://doi.org/10.34865/mb0243fase6519_w

Republished (online): 08 Aug 2025

Originally published by Wiley-VCH Verlag GmbH & Co. KGaA; https://doi.org/10.1002/3527600418.mb0243fase6519

Addendum completed: 21 Jul 2016

Published (online): 26 Jul 2018

The commission established rules and measures to avoid conflicts of interest.

2054

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MAK value documentation

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DOI: 10.1002/3527600418.mb0243fase6519

Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated the general aspects of the toxicity of inorganic fibrous dusts. As a first step, a documentation on the effects caused by inhalation of inorganic fibrous dusts and the mode of action for their toxicity was set up. For the elucidation of the mechanism of the toxicity and carcinogenicity of fibres, data derived from studies with asbestos fibres were also considered, in order to obtain some knowledge with regard to the biological effects of the other inorganic fibrous dusts. The formation of tumours in the lungs and on serous membranes is mainly the result of inflammatory processes. Chronic inflammation and cell proliferation are caused by the impairment of fibre clearance, whereby inflammation-promoting cytokines, growth factors, reactive oxygen (ROS) and nitrogen species (RNS) and chlorine radicals are released from the macrophages, inflammatory cells, alveolar cells and mesothelial cells. The generation of these radicals leads to genotoxic effects. In addition, intracellular formation of ROS and RNS can be caused by the fibres themselves, as a result of their own surface reactivity. Fibres can stimulate cell receptors and inflammasomes.
Each fibre dust group will be evaluated individually and, depending on the data available, may be classified in one of the categories for carcinogens. The Commission has already begun this procedure.

Keywords

aluminium oxide; attapulgite; calcium-sodium metaphosphate; calcium sulfate; gypsum; dawsonite; erionite; zeolite; glass wool; halloysite; potassium titanate; potassium titanium oxide; ceramic fibres; artificial mineral fibres; magnesium oxide sulfate; nemalite; brucite; palygorskite; slag wool; sepiolite; silicon carbide; rock wool fibres; wollastonite; p-aramid; mechanism of action; toxicokinetics; metabolism; genotoxicity; carcinogenicity; occupational exposure; maximum workplace concentration; MAK value; toxicity; hazardous substance

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Fibrous Dusts, inorganic

Supplement 2017

The findings from new studies published since the 1993 documentation (documentation "Fibrous Dust" 1997) have made a re-evaluation necessary. The discussion of the mechanism draws upon the MAK documentation for asbestos from 1973, 1978 and 1981 (combined in one translation: documentation "Asbestos" 1991).

The documentation for wollastonite was updated in 1996 (documentation "Wollastonite" 2001). The groups of fibrous dusts composed of glass wool and rock wool have in the meantime been broadened to include the many types of glass wool and rock wool with lower biodurability. Recent studies are available for a number of natural fibrous minerals. In addition, there are new findings on the mechanisms of action of fibrous dusts.

In the discussion of specific biological effects, this documentation also makes reference to studies of fibrous dust particles with diameters in the nanoscale range. Certain fibres such as asbestos fibres with diameters in the microscale range can split into thinner fibres because of their structural characteristics. This assessment therefore reviews the mechanistic findings on fibres with diameters in the nanoscale range. It may be necessary to evaluate fibrous nanoparticles separately.

In the following chapters, the mechanism of action of fibres is described on the basis of the current state of knowledge; findings on asbestos and other fibre types are also examined and discussed by way of comparison.

General

Definition of fibrous dust

Fibres are elongated particles with varying length-to-diameter ratios. Fibrous dusts are composed of inorganic or organic materials and are released not only from natural deposits but also as a result of industrial manufacturing and processing. The length of the fibres, but to a greater extent their diameter, determines their inhalability and thus their biological effects. Timbrell (1972) defined the aerodynamic diameter of a fibre (length > diameter) to be about three times the fibre diameter. According to international convention, which draws upon the analytical method used to determine fibre number concentrations (WHO 1997), fibrous dusts are considered toxic/damaging to the lungs in humans if their length-to-diameter ratio exceeds 3:1 and they are at the same time > 5 µm in length and < 3 µm in diameter. Fibres with other length-to-diameter ratios, in particular fibres that are ≤ 5 µm in length, are not included in the WHO definition. An important factor is the distribution of the fibre diameters in the breathing zone (documentation "Asbestos" 1991; documentation "Fibrous Dust" 1997). The relationship between fibre length and the effects of fibres is described in more detail in Section 3.1.

Overview of fibre types

The earlier documentation for fibrous dusts (documentation "Fibrous Dust" 1997) discussed inorganic and organic fibres, with the exception of asbestos. Due to their economic importance, the focus of the assessment was primarily on synthetic inorganic fibres.

The nomenclature used for synthetic inorganic fibres has been amended since the publication of the 1993 documentation. New products have in the meantime come onto the market that were not included in the earlier classification system. The following diagrams provide an overview of the classification system for fibres (see Figures 1 to 4) using the currently accepted nomenclature (classification system for fibres according to AGS 2002 and DGUV 2014). Not all of the types of fibres included in the classification system form respirable fibres.

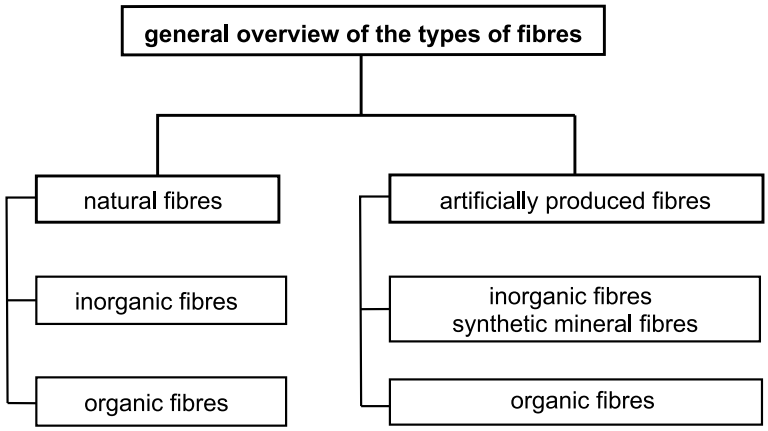

Figure 1 General overview of the types of fibres (according to AGS 2002 and DGUV 2014).

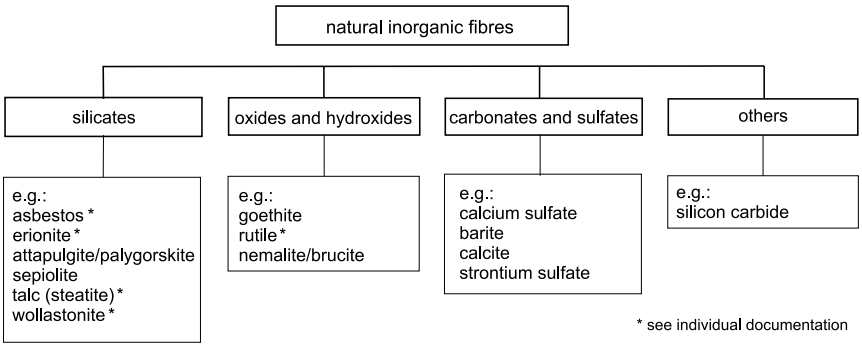

Figure 2 Classification system for natural inorganic fibres (according to AGS 2002 and DGUV 2014).

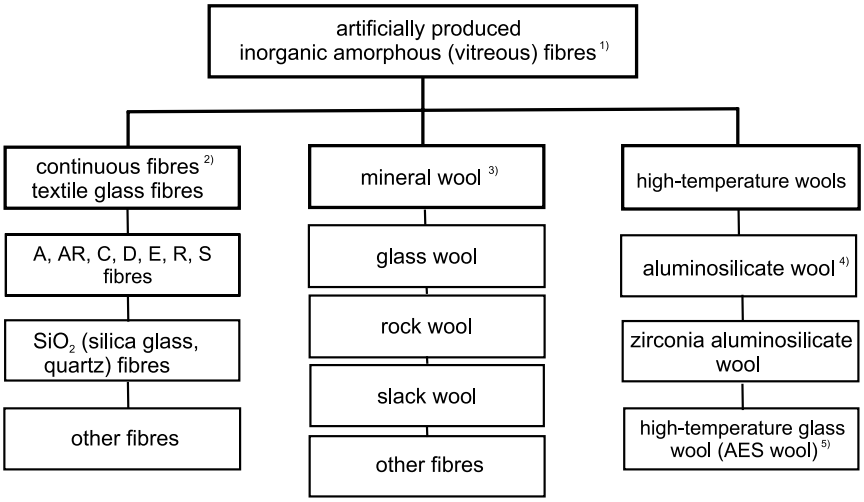

Figure 3 Classification system of synthetic inorganic amorphous fibres (according to AGS 2002 and DGUV 2014). See also the documentation for the individual fibre types.

1) Synthetic inorganic fibres include the superfine fibres (e.g. microfibres, hyperfine fibres)

2) Continuous fibres: the term is in reference to the manufacturing process. These include the short and staple fibres (produced by chopping); another name: filament fibres; A glass fibre (A = *acid resistant*); AR glass fibre (AR = *alkali resistant*); C glass fibre (C = *chemical*): fibre with increased resistance to chemicals; D glass fibre (D = *dielectric*): fibre with a low dielectric loss factor; E glass (E = *electric*), standard fibre used to reinforce plastics; R glass (R = *resistant*) and S glass (S = *strength*): fibre with increased stability and resistance to moisture

3) The term wool refers to a randomly arranged accumulation of fibres of different lengths and diameters

4) Aluminium silicate wools (ASW) were formerly referred to as ceramic fibres or *refractory ceramic fibres* (RCF)

5) AES: *alkaline earth silicates* (alkaline earth silicate wools); the high-temperature glass wools include e.g. magnesium wool, calcium magnesium wool, calcium-magnesium-zirconium-silicate wool

For the sake of completeness, the organic fibres have also been included. However, very little data are currently available for these fibres. An examination and evaluation of the data for organic fibres is planned, but will be carried out separately. With the exception of para-aramid fibres, the Commission has deferred the classification of organic fibres until a later date.

See also the documentation for the individual fibre types.

Arrangement within the solid

Crystalline: Atoms or molecules are arranged in a regular structure (crystal lattice). A diffraction pattern (reflections) is produced by interaction with X-rays.

Polycrystalline: Crystalline solid that is composed of crystalline areas (crystallites) separated by grain boundaries. A diffraction pattern (reflections) is produced by interaction with X-rays.

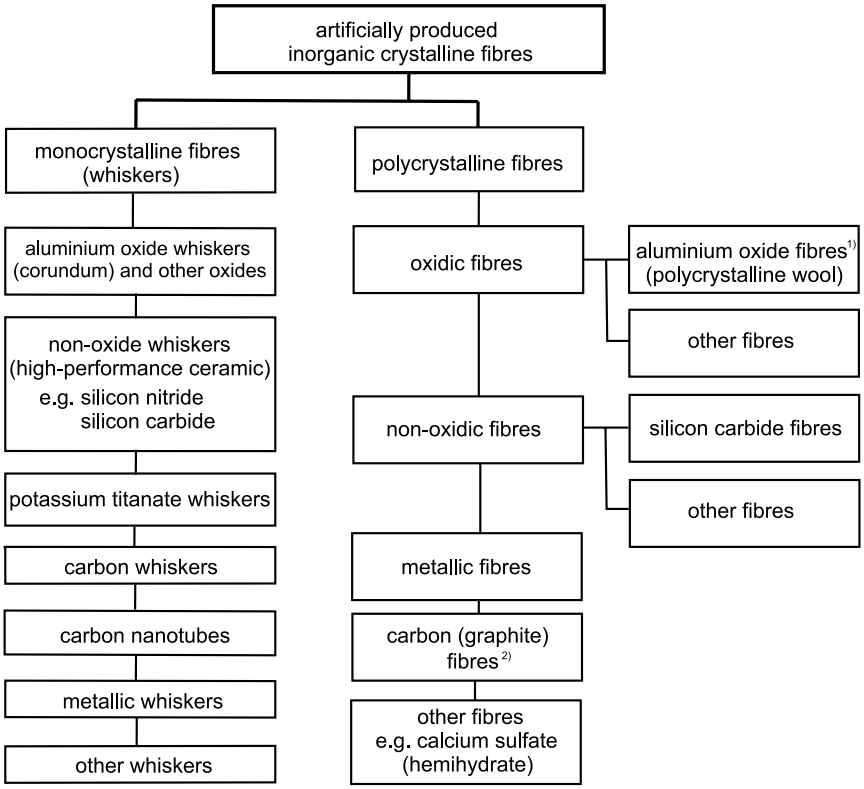

Figure 4 Classification system for syntheticartificially produced inorganic crystalline fibres (according to AGS 2002 and DGUV 2014). See also the documentation for the individual fibre types.
[1)] also belong to the group of high-temperature wools
[2)] also belong to the group of continuous fibres

Amorphous: Subcooled melt that solidified without crystallization (metastable) and softens gradually when heated; its atoms or molecules exhibit short-range order, but not long-range orientational order (crystal). No diffraction pattern (reflections) is produced by interaction with X-rays.

Synthetic inorganic fibres

Textile glass fibres (continuous fibres, filament fibres) and carbon fibres

Textile glass fibres (see Figure 3) and also carbon fibres (see Figure 4) are used for a wide range of industrial applications. In most cases, these fibres are used in the form of composites (GFK: glass fibre reinforced plastics; CFK: carbon fibre reinforced plastics). The vast majority of textile glass fibres are manufactured by drawing

molten glass through bushings to form filaments. In most cases, carbon fibres are manufactured by the pyrolysis of polyacrylonitrile following intermediate carbonization steps. The fibres that to date have proven to be technically relevant have a uniform diameter in a range from 10 to 25 μm (textile glass fibres) or from 7 to 9 μm (carbon fibres). Of these, the thoracic fraction is made up of only those fibres with a diameter in a range from 7 to 9 μm; others are deposited in the upper respiratory tract (Timbrell 1982). However, fibre chips are released during the application and mechanical processing of fibres and their composites through drilling, cutting, milling, grinding, polishing, etc.; if their dimensions are equivalent to those included in the WHO definition, they are considered to be respirable fibres.

Mineral wool (slag wool fibres, glass wool fibres, rock wool fibres)

The collective name mineral wool (see Figure 3) refers to insulation materials composed of synthetic rock wools or glass wools. Mineral wools are made up of synthetic mineral fibres; these are vitreous fibres with random orientation and a mass content of sodium, potassium, calcium, magnesium and barium oxides that exceeds 18% (AGS 2002, 2008). The chemical composition was changed between 1996 and 2000 in response to amendments to occupational safety and health guidelines. The mineral wools that were manufactured up to this time are prohibited today and are in general referred to as the "old mineral wools". A detailed assessment of different types of synthetic mineral fibres was published, for example, in IARC (2002). Data for exposure through the use of insulation wools are available in AGS (2008).

High-temperature wools

In addition to the group of fibres known as ceramic fibres (*Refractory Ceramic Fibres*, RCF), which were once the most extensively used fibres and are today referred to as aluminium silicate fibres (see Figure 3), a large number of different fibre products that are classified collectively as the high-temperature wools have been in use for many years. Essentially, this group comprises the polycrystalline fibres (a typical example being aluminium oxide wools) and the high-temperature glass fibres (AES wools). Aluminium oxide wool can be differentiated into the corundum-type (about 98% Al_2O_3, 2% SiO_2) and the mullite-type (about 72% Al_2O_3, 28% SiO_2). High-temperature glass fibres were developed for use as a substitute material in certain aluminium silicate fibre applications. The nomenclature for high-temperature wools was established in DIN EN 1094-1 (2008) and has been included in the fibre classification system shown in Figure 3 and Figure 4. Data for exposure during the manufacturing and use of high-temperature wools were published, for example, in AGS (2010).

Silicon carbide

Silicon carbide fibres (see Figure 3 and Figure 4) can be found at various workplaces also in the form of fibre chips. In addition to the technically manufactured silicon carbide whiskers (SiC whiskers), fibre chips form during mechanical processing (splitting) or the use of granular SiC. These are to be considered as respirable fibres as their particle form falls within the fibre dimensions defined by the WHO. Exposure to SiC fibre chips is in particular to be expected during the manufacture and use of abradants and abrasives of different grains.

1 Toxic Effects and Mode of Action

Inhalation and ingestion are the primary routes of absorption following exposure to fibres. Dermal absorption is negligible. However, dust deposits on the skin can lead to increased inhalation or ingestion of fibres. The amount absorbed and the depth of fibre penetration in the lungs are dependent on the length and primarily the fibre diameter; thin fibres < 3 µm in diameter can penetrate even to the alveoli.

The epidemiological data available for inorganic fibrous dusts (with the exception of asbestos and erionite) are not sufficient to assess their toxicity. Exposure data to asbestos and the resulting effects have demonstrated that in humans the target organs with malignant degeneration, are the lungs, larynx, pleura and in some cases also the peritoneum and the pericardium. In addition, the development of disease is dependent on the duration and intensity of occupational exposure, personal predisposition and the latency period (fibrosis: 15–20 years; tumours: 25–50 years). It is not possible at present to make a well-founded assessment, in particular of the carcinogenic effects, of inorganic fibrous dusts other than asbestos. There are a number of reasons for this, including the short duration of use in view of the long latency period for tumour development in humans, the lower doses compared with those resulting from exposure to asbestos, the smaller number of exposed persons, and concurrent causes of tumour development.

After exposure to aluminium silicate fibres, non-malignant pleural changes, such as pleural plaques, and fibrotic, in some cases also calcified, thickening of the pleural membrane, were observed in humans.

Animal studies and in vitro studies have demonstrated a broad spectrum of fibre properties that are potentially hazardous to the health after exposure. These are dependent upon the characteristics of the fibres. After inhalation exposure, some fibres are removed only very slowly from the respiratory tract by clearance. A long clearance half-life and the repeated uptake of persistent fibres can lead to a chronic response of the respiratory tract and lung tissue and ultimately to fibrosis and tumours (lung cancer, mesothelioma). A number of different fibres, such as silicon carbide fibres, aluminium silicate fibres (RCF) and persistent special-purpose fibres, induce inflammatory responses, fibrosis of the parenchyma of the lungs, pleural lesions and tumour development, in particular lung tumours and mesotheliomas; these effects were found primarily in rats and hamsters (exposure: inhalation, intratracheal, intraperitoneal, intrapleural). Slag wool fibres and rock wool fibres and biosoluble glass fibres do not result in comparable carcinogenic effects, but rather in only inflammatory changes.

Exposure to silicon carbide whiskers led to pneumoconiosis with nodular changes similar to silicosis in the lungs; however, co-exposure to granular silicon carbide particles cannot be ruled out. Other adverse health effects that have been reported as resulting from exposure to aluminium silicate fibres (RCF) or silicon carbide whiskers are irritation of the respiratory tract, including dyspnoea and bronchial obstruction (asthma, chronic bronchitis, emphysema), and irritation of the eyes and skin.

2 Mechanism of Action of Fibres

Fibres that have reached the lungs can be eliminated by mucociliary clearance from the trachea and bronchi and by macrophage-dependent clearance from the smaller bronchioles and the alveoli; the macrophages take up the foreign substances and transport them to the mucociliary escalator by amoeboid movements. Persistent long fibres remain in the lungs for a longer period of time because long fibres first have to be broken up into smaller pieces before they can be engulfed by phagocytosis. The local dose of persistent long fibres is thus markedly higher than that of short fibres. The greater lung burden leads to the migration of monocytes from the bloodstream into the lung tissue; these differentiate to phagocytes in the lung tissue and support the local macrophages in their cleaning function. The release of inflammatory mediators by the phagocytes and the mobilization of other inflammatory cells—in particular neutrophilic granulocytes—ultimately leads to the development of hypochlorite (HOCl), reactive oxygen species (ROS) and reactive nitrogen species (RNS) and to inflammatory damage of the tissue in overloaded lung areas. Granulocytic infiltration into the inflamed lung area is the primary indicator of the transition from mere physiological burden to the pathological condition of inflammation. This also constitutes a proliferation stimulus for type II alveolar epithelial cells, which can develop into lung tumours.

However, the cleaning system of the lungs reaches the limits of its effectiveness because of the particular dimensions of fibres; as a result, additional quantitative and qualitative pathomechanisms are involved:

- During inhalation, more fibres than granular particles can be deposited in the respiratory tract because fibres are more likely to adhere to the surface of the respiratory tract (interception).
- Due to their elongated form, it is more difficult for the mucociliary escalator to remove fibres than granular particles. Phagocytes cannot engulf and remove long fibres that are longer than the diameter of phagocytes. Before they can be engulfed by the phagocytes, these fibres first have to be broken down either mechanically or through the effects of the surrounding environment. The mechanical stability and biodurability of the fibres play an important role in this. A particular property of asbestos fibres in this respect is their tendency to break longitudinally rather than transversely after mechanical stress.

Unlike after exposure to granular particles, excessive volume is not the primary condition leading to macrophage overload after exposure to fibres. Long and thin fibres that cannot be engulfed completely lead to "frustrated phagocytosis" during which accumulation of macrophages along the fibre and increased cell death were observed (Dörger et al. 2000, 2001; Hamilton et al. 2009; Ji et al. 2012; Murphy et al. 2012, 2013; Poland et al. 2008; Ye et al. 1999, 2001). Other pathophysiological effects are:

- Rigid and biodurable fibres can mechanically damage macrophages and epithelial cells, thereby leading to increased cell death and a stimulus for epithelial cell proliferation.
- Mechanical interaction with the mitotic spindle apparatus and interference with chromosomes were described for asbestos fibres in vitro. The role this phenomenon plays in carcinogenicity is still unclear.

- Fibres break through the barrier of the epithelial cells and penetrate to the pulmonary interstitium, where they stimulate fibrotic processes via fibrogenic mediators.
- Due to their rigidity and the impairment of clearance, persistent fibres can be translocated after absorption by inhalation, not only into the interstitial lung tissue, but even into the pleural cavity and, although less frequently, into the pericardium and peritoneum.

Clinical sequelae of the pathological changes induced by fibres are chronic inflammation of the respiratory tract and the lungs, pulmonary fibrosis and lung cancer. A distinctive feature, which has been observed to date only for some fibrous particles, but not for granular particles, is the induction of the characteristic hyaline, and in some cases calcified, thickening of the pleural wall, pleural plaques and mesenchymal tumours (mesotheliomas) of the serous membranes, pleura, pericardium and peritoneum.

The prevailing opinion today is that the predominant pathogenic mechanisms are chronic inflammatory processes that are primarily determined by the "three d's: dose, durability, dimension", that is, the inhaled dose and the local dose, the biodurability and the fibre dimensions (length, diameter). Other key factors are the chemical composition of the fibre surface and its rigidity and straightness. The number of fibres is considered to be a suitable measure of exposure. This differs from the usual use of the term dose in toxicology, which refers to the mass of a substance.

Simultaneous exposure to fibres and particles can lead to combination effects. Biopersistent granular particles may also inhibit clearance and lead to chronic inflammation, fibrosis and lung tumours. These effects are intensified by the additional presence of fibres in the lungs that are difficult to remove because of their length and potential biopersistence (IARC 2002, 2009; NTP 2010, 2016).

2.1 Non-malignant fibre-induced diseases

Non-malignant fibre-induced diseases affect the pulmonary interstitium and the pleura. In the case of the pleura, toxicological investigations have focused on the translocation and the retention of fibres in the pleural cavity, while in the case of lung fibrosis, the focus has been on general aspects of fibrogenesis or the description of cellular and molecular mechanisms. The following provides a summary of the most important findings.

2.1.1 Lung fibrosis

Lung fibrosis is the formation of scars and excess connective tissue in the pulmonary interstitium with an accumulation of collagen in the alveolar septa, which leads to a reduction in elasticity. It is considered the final stage of chronic inflammatory interstitial lung disease (Pschyrembel 2013). Certain types of lung fibrosis (pneumoconiosis) develop as a result of the chronic lung burden and overloading of clearance mechanisms after exposure to biopersistent particles and fibres; neighbouring tissue cells, such as alveolar epithelial cells, are damaged or replaced by connective tissue.

Findings for asbestos and erionite fibres

Asbestosis is defined as bilateral diffuse interstitial lung fibrosis. Lung fibrosis is induced also by erionite (Baris et al. 1987; Kliment et al. 2009). As asbestosis develops very slowly and, in most cases, only at high levels of exposure (5 to 20 times the number of (amphibole) fibres in the lungs in comparison with asbestos-associated pleural changes), a dose–response relationship is to be assumed (Mossman and Churg 1998). Numerous studies of the complex fibrogenic mechanism revealed that it involves the participation of a large number of different cell types and is characterized by a persistent inflammatory response that involves the generation of ROS, growth factors, chemokines and cytokines. In addition, a large number of signalling cascades are triggered involving mitogen-activated protein kinases (MAPKs) and the transcription factor NF-κB. Transcription factors such as NF-κB and activator protein 1 (AP-1) are activated, which upregulates genes which govern proliferation, apoptosis and inflammation in the lungs (reviews of Mossman and Churg 1998; Robledo and Mossman 1999; Rosenbloom et al. 2010).

Findings for other mineral fibres

A number of studies revealed that silicon carbide fibres had a fibrosing effect in humans. Microscopic investigation of the tissues from 3 workers exposed to silicon carbide found, among other things, nodular fibrosis containing silicon carbide bodies and "ferruginous bodies", a large amount of carbon pigment and interstitial fibrosis (Massé et al. 1988). In a cohort study of workers of a silicon carbide smelter, the standardized mortality ratio for pneumoconiosis was significantly increased (Romundstad et al. 2001). The authors assumed that the pneumoconiosis was caused by many years of exposure to silicon carbide fibres and crystalline silicon carbide. A study that examined the lung tissue of 15 workers (post mortem) who were employed for 23 to 32 years in the silicon carbide industry reported 6 cases of lung fibrosis and 4 other cases of both lung fibrosis and lung cancer (Dufresne et al. 1995). In addition to silicon carbide particles, a large number of silicon carbide fibres (length > 5 μm; 39 300 fibres/mg dry lung tissue) were found in the lung tissue of a worker from a silicon carbide plant (term of employment 42 years) who had been diagnosed with silicon carbide pneumoconiosis (Dufresne et al. 1993).

A case study reported on a 41-year-old man who had been diagnosed with interstitial fibrosis. Particles and fibres were found in the lung tissue. The fibres were analysed chemically and identified as glass wool fibres. The man had been exposed to glass wool fibres over a period of many years (Guber et al. 2006).

There are no reliable findings of the fibrosing effect of other mineral fibres such as slag wool and rock wool fibres and aluminium silicate fibres (RCF) in humans; the majority of the findings are negative. In contrast, a number of animal studies found that exposure to these fibres led to lung fibrosis (IARC 2002; Montelius 2005; NTP 2010; EU 2011).

2.1.2 Pleural fibrosis

A distinction is made between circumscribed pleural fibrosis (pleural plaques) and diffuse pleural fibrosis (diffuse pleural thickening). Pleural fibrosis in general re-

sembles fibrosis in other organs and can be defined as an excessive deposition of matrix components. These pleural changes are characterized by inflammation and impaired fibrin metabolism, which maintains the fibrotic process. It is assumed that the complex interaction between subpleural fibroblasts, mesothelial cells and inflammatory cells as well as the release of profibrotic mediators, coagulation factors and fibrin contribute to the development of fibrinogen in the fibrotic process. In addition to cytokines and growth factors, also reactive oxygen species play a role in fibre-induced fibrogenesis (Huggins and Sahn 2004).

Diffuse pleural fibrosis

This type of pleural fibrosis is interpreted to be the result of benign asbestos-induced pleural effusions. Diffuse pleural fibrosis is a thickening of the pleura consisting of collagenous connective tissue. This can occur in varying degrees of severity, up to coverage of the entire lung surface (Greillier and Astoul 2008). Diffuse pleural changes occur less frequently than pleural plaques, are generally unilateral, rarely calcify and, in contrast to pleural plaques, can affect the interlobular fissures (overview in Greillier and Astoul 2008; Fletcher and Edge 1970). Diffuse pleural changes can cause the adhesion of the parietal and visceral pleura, resulting in functional impairment.

Circumscribed pleural fibrosis (pleural plaques)

This type of pleural fibrosis affects the parietal pleura. Pleural plaques are thickenings of the parietal pleura made up of collagenous connective tissue which can calcify over time. They have a fibrous structure and limited fibroblastic cellular activity. Pleural plaques occur almost always bilaterally and occur as hyaline fibrotic lesions; the plaques are made up of acellular bundles of collagen in wavy, basket-weave patterns. The latency period after exposure to asbestos is in most cases 20 to 30 years (Greillier and Astoul 2008). Pleural plaques generally lack functional relevance (Pairon et al. 2013, 2014) and are considered to be a marker of exposure to fibres.

Findings for asbestos and erionite fibres

Fibres such as erionite, and amphibole and serpentine-type asbestos can induce pleural plaques. Pleural plaques were found in up to 60% of exposed persons, while they rarely occur in the general population of countries without natural exposure to asbestos (Pairon et al. 2013).

A number of authors (Harber et al. 1987; Weiss 1993) concluded in their studies that pleural fibrosis is a marker of exposure to fibres. Even after adjusting for cumulative exposure to asbestos, prospective studies found an association between pleural plaques and bronchial carcinoma (Pairon et al. 2014) and pleural plaques and pleural mesothelioma (Pairon et al. 2013). According to the findings of these studies, pleural plaques are not merely an exposure marker, but rather an independent risk factor for malignant end points induced by exposure to fibres. A review drew attention to the difficulties inherent in determining exposure to fibres and again suggested that pleural plaques are primarily a marker of exposure (Maxim et al. 2015). In view of this, a conclusive answer has yet to be found as to whether pleural plaques should be considered an independent risk factor for malignant diseases.

Findings for other mineral fibres

Like asbestos and erionite fibres (Gräsel et al. 2008; IARC 2009; Selçuk et al. 1992) and unlike other types of fibres, aluminium silicate fibres (RCF) induce pleural plaques in humans (ATSDR 2004; IARC 2002; Lemasters et al. 1994; Lockey et al. 1996, 2002, 2012; NIOSH 2006, 2011; Trethowan et al. 1995; Utell and Maxim 2010). A significant relationship between pleural plaques and increasing exposure to aluminium silicate fibres was found in the studies of Lockey et al. (2002), Lentz et al. (2003) and Lockey et al. (2012). This relationship was specified to apply to the categories "cumulative exposure", "cumulative pulmonary dose of all fibres" and "cumulative pulmonary dose of fibres of < 0.4 μm in diameter and < 10 μm in length" (according to the authors, fibres of this size migrate to the parietal pleura) (Lentz et al. 2003). A follow-up study revealed that fibres were still found in the lung tissue of exposed workers 13 to 20 years after exposure to aluminium silicate fibres (RCF). In addition, there was a significant correlation between the time span (beginning of exposure to aluminium silicate fibres to the time of examination) and the occurrence of pleural changes (OR: 10.4, 95% CI 3.0–36.3 and OR: 10.8, 95% CI 2.4–47.9) in workers with a term of employment > 20 years or a latency period of > 20 years (Lockey et al. 2012).

It should be noted that pleural plaques only began to be detected with greater frequency after the introduction of the more sensitive method of high-resolution computed tomography (HRCT) (Fireman 2014), which is why there is relatively little epidemiological evidence available from the period before the 1990s.

2.2 Malignant fibre-induced diseases

2.2.1 Lung carcinomas

Asbestos-induced lung cancer can be classified in the same main histological classes as those observed in persons not exposed to asbestos, i.e. epithelial, adenomatous, large-cell, small-cell, sarcomatoid and adenosquamous tumours (Oksa et al. 2014).

Findings for asbestos and erionite fibres

Epidemiological investigations have shown that both asbestos fibres and erionite fibres can cause lung tumours. The carcinogenic effects of asbestos fibres and erionite fibres observed in humans have unanimously been confirmed in animal studies after exposure by inhalation and intrapleural and intraperitoneal administration (documentation "Asbestos" 1991; documentation "Fibrous Dust" 1997; IARC 2009).

Findings for other mineral fibres

A number of animal studies found that certain types of synthetic mineral fibres can cause lung tumours in rodents after intratracheal, intraperitoneal and intrapleural administration (documentation "Fibrous Dust" 1997; IARC 2002; NTP 2010, 2016; see also the documentations for the individual fibres).

2.2.2 Mesotheliomas

In most cases, a mesothelioma is a diffusely growing tumour that develops from the single-layer squamous epithelium of the mesothelium of the serous membranes. The most common area affected by this type of tumour is the parietal pleura. Mesotheliomas are often difficult to diagnose. The latency period associated with pleural mesotheliomas is up to 40 years.

Findings for asbestos and erionite fibres

Asbestos fibres and erionite fibres induce mesotheliomas in exposed persons. The incidence of mesotheliomas in rats and mice was increased after exposure to erionite (documentation "Asbestos" 1991; documentation "Fibrous Dust" 1997; IARC 2002, 2009).

Erionite is more potent with respect to the induction of mesotheliomas than any of the asbestos types. The most common area affected is the parietal pleura of the thoracic cavity (Baris et al. 1996; IARC 2009).

Findings for other mineral fibres

Certain types of glass fibres, rock wool fibres, silicon carbide fibres and ceramic fibres may induce malignant mesotheliomas in rodents after exposure by inhalation and intratracheal and intraperitoneal administration (documentation "Fibrous Dust" 1997; IARC 2002; NTP 2010, 2016; see the documentations for the individual fibres).

Most of the epidemiological studies yielded negative results for an association between exposure to synthetic mineral fibres and the diagnosis of mesotheliomas or lung tumours. However, these results are to be interpreted with caution, as the level of exposure in the studies of synthetic mineral fibres carried out to date was markedly lower than in epidemiological studies of asbestos that yielded positive findings. Furthermore, the observation periods of the epidemiological studies of synthetic mineral fibres were in some cases not yet long enough to be able to draw reliable conclusions at this time, as the latency periods are assumed to be comparable in length to those after exposure to asbestos (Boffetta et al. 2014; IARC 2002, 2009; ILSI 2005; Lipworth et al. 2009; NTP 2010, 2016).

2.3 Mechanisms of the toxicity and carcinogenicity of fibres

The mechanism of fibre toxicity and carcinogenicity is very complex and many of the details still need to be clarified. The primary pathways that have been suggested for fibre-induced carcinogenicity are based on a) the physical and chemical properties of the fibres (including fibre dimensions, chemical composition, stability, surface reactivity, biopersistence) and b) the inflammatory response that is triggered in the lungs after inhalation of the fibres (Hei et al. 2006; IARC 2009; WHO 2006) (see Figure 5).

Even though asbestos is not the focus of the mechanistic considerations discussed in this section, the extensive insights obtained as to the effects of exposure to asbestos are used as a point of reference to determine to which extent synthetic mineral fibres induce similar effects.

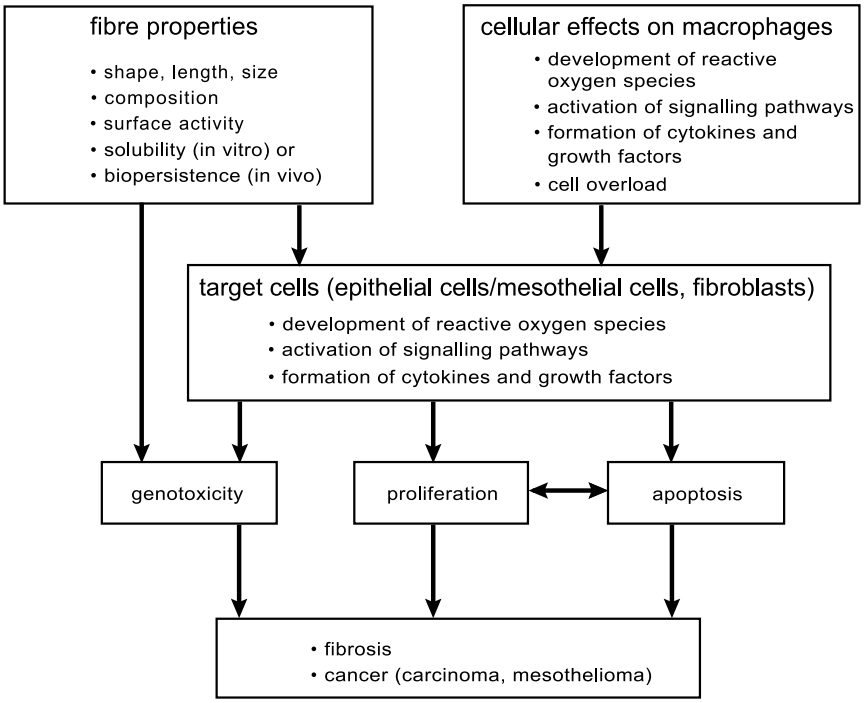

Figure 5 Mechanism of fibre toxicity (modified according to Nguea et al. 2008 from NTP 2010)

Accordingly, a number of areas of cytological activity can be identified that contribute to the development of cancer.

- Natural and synthetic mineral fibres are able to form reactive oxygen species and reactive nitrogen species (ROS, RNS) and halogen intermediates in acellular and in vitro model systems. These include H_2O_2, $O_2^{\bullet-}$, singlet oxygen, peroxynitrite, nitronium ions and HOCl (Halliwell 2006) and can cause a broad spectrum of DNA modifications. Gene mutations and gene amplifications have also been described (Wang et al. 1999). Incomplete or "frustrated" phagocytosis leads macrophages to release messengers that first attract other macrophages and finally neutrophilic granuloctyes (neutrophils). Activated neutrophils release cytotoxic HOCl by means of a myeloperoxidase-induced reaction (Parker et al. 2012) and other reactive substances as by-products, including reactive oxygen species and reactive nitrogen species. The latter can affect neighbouring cells and also cause DNA damage.
- Fibres can lead macrophages, mesothelial cells and alveolar epithelial cells to an inflammasome-mediated release of cytokines and growth factors, which results in chronic inflammation and cell proliferation in the lungs and pleura (Dostert et al. 2008).

Fibres can react directly with target cells (epithelial cells, mesothelial cells, fibroblasts) by inducing inflammatory signals or chromosomal changes (Hart et al. 1994; NTP 2010, 2016; Ong et al. 1997; Zhong et al. 1997) after receptor-mediated endocytosis (Heintz et al. 2010). Aneuploidy, micronuclei and chromosomal aberrations have been observed.

In particular, long, thin asbestos fibres can also directly interact with the mitotic spindle by snagging chromosomes or blocking their movements (Ault et al. 1995).

Fibres can induce epigenetic changes, i.e. hereditary changes in gene expression that are not anchored to a change in the DNA sequence (Andujar et al. 2007; Lecomte et al. 2005).

However, it is not clear how the different overlapping cellular signalling pathways that are involved with gene activation and inactivation are coordinated, because the networks of inflammation, DNA repair, cell proliferation and apoptosis interact with each other. It is well substantiated that prolonged inflammation is closely related with the development of tumours (Bernstein 2007 a, b; Huang et al. 2011; ILSI 2005; WHO 2006).

While reactive oxygen species are produced by both non-fibrous and fibrous particles, cell cycle-associated chromosomal and nuclear anomalies appear to occur as a result of exposure to fibres.

The mechanisms of fibre-induced tumour development that were briefly mentioned above are discussed in more detail in the following.

2.3.1 Surface reactivity of fibres

Surface reactivity plays an important role in the pathogenic effects of fibres (documentation "Fibrous Dust" 1997). The interaction of the fibre surface with O_2 and with biomolecules from the physiological environment and cell membranes can lead to the generation of reactive substances such as ROS and RNS, which may cause such effects as pulmonary inflammation and increase the inflammatory response (Braakhuis et al. 2014). Surface reactivity is determined by chemical composition, the structure of the fibre surface and by the physico-chemical properties of the fibre surface, such as hydrophilicity, charge, morphological composition and the ability to exchange catalytically active metal cations. This has an impact on the following effects (Bernstein et al. 2005; Braakhuis et al. 2014; Fubini et al. 1998; Hippeli et al. 2001; Kane et al. 1996; Kucki et al. 2014; Monchaux et al. 1981; Warheit and Gavett 1993; WHO 2008):

- Adsorption to exogenous and endogenous molecules, which influences not only the formation of a complex protein corona and receptor-mediated cell internalization, but also the potency and mechanism of action
- Adhesion to the cell membrane and internalization by non-phagocytic cells
- Internalization by phagolysosomes and thus an increased release of cytokines and growth factors
- Release of ROS and RNS and DNA damage
- Release of metal ions
- Biopersistence and clearance
- Translocation into various compartments

Atoms and ions are more weakly coordinated on the fibre surface and therefore exhibit greater reactivity (Fubini 1993, 1997; Hochella 1993; Kucki et al. 2014). Different reactions are caused by transition metal ions on the fibre surface. They can be oxidized, hydrated and hydroxylated in air (Fubini 1997). Electrostatic interactions and electrochemical activity on the fibre surface can lead to impairment of intracellular processes. The transfer of ions between the fibre surface and the biological environment can change the surface charge and thus the pathogenicity (Fubini and Otero-Aréan 1999). Transition metals can occur in amorphous synthetic fibres and carbon nanotubes both in trace amounts and also in quantitatively relevant amounts (see Table 1).

Their metal content makes redox reactions possible (Aust et al. 2011).

Table 1 Quantitative amounts of metals in different fibres (Cavallo et al. 2004; De Vuyst et al. 1995; Fubini and Otero-Aréan 1999; Hippeli et al. 2001; Sanchez et al. 2009)

Fibre	Metal	Mass percentage
glass wool fibres	iron oxide	0–1
	zirconium oxide	0–0.4
	magnesium oxide	3
special-purpose fibres	iron oxide	0–0.4
	zirconium oxide	0–4
rock wool fibres	iron oxide	1.5–12.4
	magnesium oxide	9.25–10.9
	manganese oxide	0–0.16
slag wool fibres	iron oxide	0.9–5
aluminium silicate fibres	iron oxide	0–1
	zirconium oxide	0–17
	yttrium oxide	0–8
carbon nanotubes	cobalt, iron, nickel, yttrium, molybdenum	traces

Endogenous iron may also be adsorbed to the fibre surface or into the surface pores. However, this has been observed to date only for erionite (Eborn and Aust 1995; Sanchez et al. 2009).

Fibres with surface silanol groups have a negative surface charge and can thus adsorb endogenous iron (Dugger et al. 1964; Olson and O'Melia 1973; documentation "Fibrous Dust" 1997). Fibres with surface oxygenic functional groups have a similar potential for adsorbing metal cations due to their negative polarity and charge (Crumbliss and Garrison 1988; Ghio et al. 2004). Furthermore, the iron (III) ion reacts with silanol groups to form an iron silanol complex. Biological sources of iron and other transition metal ions occur intracellularly and extracellularly. Endogenous stores include ferritin, haemosiderin, aconitase, xanthine oxidase and iron-sulfur cluster proteins, which have bound Fe^{2+}/Fe^{3+} as additional iron ligands (ferredoxins) via cysteine residues (Aust et al. 2011; Breuer et al. 1995; Koerten et al. 1990). The

extracellular space is an additional reservoir for iron (III) ions, that are bound for example to transferrin, lactoferrin and ferritin.

An increase in the concentration of ROS and RNS can, on the one hand, be a direct result of the generation of ROS on reactive fibre surfaces (Churg 2003; Donaldson et al. 2013; Fantauzzi et al. 2012; Oberdörster 2002; Gazzano et al. 2005) and, on the other hand, be an indirect result of the activated defence mechanisms after exposure to fibres.

A correlation was found between the amount of coordinated active iron ions on the surface and the formation of ROS in vitro (Fach et al. 2002; Fubini et al. 1995; Prandi et al. 2001) but not with the degree of DNA damage (Churg et al. 2000). This is plausible because, on the one hand, DNA damage can also be caused by other reactive substances and, on the other hand, ROS can induce other effects as well (Dizdaroglu et al. 2015; Jaurand 1997).

With respect to the role of fibre associated metal ions and their redox activity in the formation of ROS and RNS, it was observed that no increased reactivity of multiwall carbon nanotubes (MWCNT) due to iron and other electrochemically active material could be observed that could implicate carcinogenicity of nanotubes (Donaldson et al. 2010). In contrast, it was demonstrated that metal catalyst residues are bioavailable in nanomaterials under physiological conditions (Guo et al. 2007; Liu et al. 2007; Shvedova et al. 2005). The findings reported by individual studies of carbon nanotubes (CNTs) were dependent on the specific synthesis of the materials, their exact composition and the bioavailability of the metal ions used. In vitro studies showed that long needle-shaped MWCNTs (*diameter* = 74 nm, *length* = 5.7 µm; *diameter* = 64 nm, *length* = 4 µm) led to the dose-dependent development of as yet unidentified radicals in both the presence and absence of BEAS-2B cells (Nymark et al. 2014).

2.3.2 Production of reactive oxygen and nitrogen species (ROS, RNS)

Numerous studies have demonstrated the production of ROS and RNS by synthetic mineral fibres such as glass wool, rock wool, slag wool, silicon carbide whiskers and ceramic fibres in both cell-free systems and cell model systems (IARC 2002; ILSI 2005; Nishiike et al. 2005; NTP 2010; Rapisarda et al. 2015; WHO 2006).

The following mechanisms are proposed for the generation of ROS and RNS:

1. the direct formation of activated oxygen species (hydrogen peroxide, superoxide anions, hydroxyl radicals) on the fibre surface (Maples and Johnson 1992)
2. following uptake of the fibres into the target cells: generation and release of the activated oxygen species, accelerated by catalytically active iron (II) ions. Depending upon the affinity of the fibre surface for iron ions, the fibres extract these ions from the loosely bound iron-sulfur centres (so-called 4Fe-4S clusters) of certain enzymes (e.g. dehydratases) (Liochev and Fridovich 1999). A high concentration of reactive oxygen species induces a metabolic state in the cell that is known as oxidative stress (Cardinali et al. 2006).

Aggressive oxygen and nitrogen species are released ("oxidative burst") as soon as phagocytes (macrophages, monocytes and polymorphonuclear granulocytes) more or less fully engulf fibres and attempt to degrade them. Particularly for longer fibres,

elimination from the lungs proceeds at a slower rate than is the case for biopersistent granular dusts. This triggers the migration of additional macrophages in the lungs, which results in an increase in ROS and RNS levels and more extensive inflammatory damage to the tissue (Warheit and Gavett 1993). In addition, there is a marked increase in superoxide production by macrophages at a fibre length of > 6 μm and above (Ohyama et al. 2000, 2001).

Also involved in the generation of reactive oxygen species is membrane-bound NADPH oxidase, which supplies the primary product $O_2^{\bullet-}$ for other toxic metabolites. ROS are released both into the lumen of phagolysosomes and into the extracellular environment. During an "oxidative burst", the formation of ROS are accompanied by that of RNS, in particular nitric oxide (NO); which reacts further with ROS to form peroxynitrite or nitronium ions as a secondary product (Beckman and Koppenol 1996; Felley-Bosco 1998; Halliwell 2006; Pacher et al. 2007). Nitric oxide and thus also its secondary products form during phagocytosis as a result of the stimulation of inducible NO synthase (iNOS). Myeloperoxidase is also involved, an enzyme that is most abundantly expressed in neutrophilic granulocytes. It forms hypochlorite anions (OCl^-) from hydrogen peroxide and chloride; these undergo homolytic dissociation to form $Cl^\bullet$ and can chlorinate nucleobases in DNA, for example (Hawkins et al. 2007).

DNA damage is the primary effect of the release of reactive oxygen species and reactive nitrogen species. Strand breaks are induced by modifications to deoxyribose; in addition, chemical changes or destruction of the various bases lead to mismatches and mutations. The formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-OH-dG) has been reported frequently, as were a large number of other DNA base modifications with varying potential for base mismatches (Cooke et al. 2003; Evans et al. 2004). Oxidative stress is an important mechanism of the carcinogenicity of fibres (Nguea et al. 2008). Carbon nanotubes, both single-wall (SWCNTs) and multi-wall (MWCNTs), increased the level of ROS, for example in the transformed mouse macrophage cell line RAW 264.7 (Di Giorgio et al. 2011).

2.3.3 Interaction with endogenous biomolecules and non-phagocytizing cells

Endogenous materials can be adsorbed to the fibre surface by non-covalent bonds and form a corona. This is dependent on chemical composition, charge, polarity, the potential for forming hydrogen bonds and hydrophobicity (Fubini 1997; IARC 2009). The binding of fibres to cells and the internalization of fibres and their toxicity in non-phagocytizing and phagocytizing cells has been found to be influenced by the binding of phospholipids, proteins and cations and their release (Boylan et al. 1995; Churg 1996; Fubini et al. 1995; Kucki et al. 2014; Møller et al. 2014).

Therefore, the adsorption of vitronectin via interaction with integrin receptors can lead to the internalization of fibres (crocidolite, chrysotile, but not wollastonite, in spite of its negative charge) by non-phagocytizing cells such as mesothelial cells (Boylan et al. 1995; Fubini et al. 1998).

The internalization of asbestos fibres was first described in the 1970s (Jaurand et al. 1979; Suzuki 1974) and later confirmed by a number of other authors (Boylan et al. 1995; Pande et al. 2006; Wu et al. 2000). All of the authors agree that the internaliza-

tion of asbestos fibres is intensified by their interaction with the membrane proteins adsorbed to the fibre surface. Adsorbed vitronectin (a serum protein) was found to increase internalization via the integrin $\alpha_V\beta_5$ receptor. Other studies reported that internalized asbestos fibres were surrounded by a marker of endosomes and phagosomes, the small GTPase Rab5a (Broaddus et al. 2011; Henry et al. 2004; Nagai et al. 2011). These findings explain the mechanism by which asbestos is internalized by the cell via endocytosis.

Similar to the case with asbestos fibres, synthetic mineral fibres that are not removed by macrophages can be internalized by epithelial cells and transported to the pleural space (NTP 2016; Oberdörster 2002).

Fibres can interact with the cells of the alveoli and bronchi and those of the parietal pleural membrane, leading to damage of these cells. Exposure to various fibres, such as glass wool and rock wool fibres, aluminium silicate fibres and crocidolite, leads to a dose-dependent reduction in the number of microvilli in human mesothelial cells. Increasing crocidolite concentrations result in a change of the cell shape and the formation of holes and tears in the cell membrane. A rock wool fibre type at elevated concentrations of 15 and 20 µg/cm^3 caused the formation of protrusions or blebs on the cells as a marker of oxidative stress.

In a few cases, this was observed also after exposure to glass wool fibres. In these cases, the fibres had already been partially phagocytized into the cells (Cavallo et al. 2004). According to the authors, this was caused by the cytotoxic effects of the fibres, or rather the mechanical effects of the fibres on the cells.

Carbon nanotubes As the importance of carbon nanotubes (CNTs) for industrial applications has grown exponentially over the past few years, a large number of studies have been carried out to examine how these fibres interact with endogenous biomolecules and non-phagocytizing cells.

Depending upon their length, carbon nanotubes (single-wall carbon nanotubes, SWCNTs, and multi-wall carbon nanotubes, MWCNTs) can be internalized by non-phagocytizing cells such as endothelial cells, mesothelial cells, epithelial cells and T cells; once internalized, they are present within the cytoplasm either membrane-bound or non-membrane bound (Donaldson et al. 2006; Møller et al. 2014). A number of studies found that large non-functionalized CNTs cannot enter the cells via endocytosis. While these fibres can penetrate to the cell as a result of the conjugation of folic acid with MWCNTs, probably via a receptor-mediated pathway (Dhar et al. 2008; Kam et al. 2005 a; Kang et al. 2008, 2009, 2010), this was not possible for MWCNTs coated with albumin. The presence of certain additional ligands accelerates and facilitates the endocytosis of oxidized nanotubes (Bhirde et al. 2009; Iancu et al. 2011; Kam and Dai 2005; Kam et al. 2005 b; Kang et al. 2008). The surface coating plays a significant role in the uptake of CNTs into cells. The formation of a protein corona and the adsorption of receptor-specific proteins can enable the internalization of fibres into non-phagocytizing cells (Møller et al. 2014).

Therefore, CNTs can directly penetrate cells if they are small in diameter and have a positively charged surface. The presence of specific ligands can facilitate the uptake of large-diameter CNTs, as these are taken up by the cells via ligand-mediated endocytosis.

Straight, elongated MWCNTs are not engulfed via endocytosis, but directly penetrate the plasma and nuclear membrane; this is dependent upon diameter and rigidity (Hu et al. 2010; Nagai et al. 2011). The presence of membrane-bound intracellular fibres can be explained, however, by an active internalization process; this was reported in a number of studies (Foldbjerg et al. 2014; Guo et al. 2011; Lindberg et al. 2013; Manshian et al. 2013; Monteiro-Riviere et al. 2005; Srivastava et al. 2011).

A number of recent studies found that rigidity and a straight, needle-like shape are critical determinants of the pathogenic effect of CNTs. In contrast to flexible MWCNTs ("*long tangled*", diameter ≥ 50 nm, length around 13 μm), inflexible and straight MWCNTs ("*long needle-like*", diameter 8–15 nm, length 10–50 μm) can cause cell and DNA damage because of their rigidity and needle-like shape. These effects have been found in female C57Bl/6 mice after exposure by inhalation and after pharyngeal application in the lung cells and cells from bronchial lavage (FIOH 2013). Effects on the lung tissue that were dependent on fibre length were observed in mice exposed to different types of MWCNTs (varying lengths: 1–2 μm straight, 1–5 μm tangled, 36 μm straight) by aspiration (Murphy et al. 2013). Acute neutrophilic inflammation is induced only by long fibres; this can be determined by bronchoalveolar and pleural lavage. In addition, increased thickening of the alveolar septa and lesions along the parietal pleura were observed. After intraperitoneal injection in rats, it was found that a number of different straight CNTs have a strong mesotheliomainducing effect, even when compared with crocidolite (Rittinghausen et al. 2014).

Interaction between fibres such as asbestos and carbon nanotubes (Nagai and Toyokuni 2012) and mesothelial and epithelial cells causes both damage to the surrounding tissues and fibrotic lung diseases and pleural damage. According to Nagai and Toyokuni (2012), cellular uptake of the fibres is an important step in the carcinogenic process.

2.3.4 Chronic inflammation

In general, chronic inflammation can lead to the initiation, promotion and progression of tumours (de Visser et al. 2005). Chronic inflammation is considered the driving force behind further pathophysiological cellular and tissue changes in the lungs (IARC 2002; ILSI 2005; NTP 2010). Epithelial cell damage leads to hyperplasia and hypertrophy and occasionally to the development of tumours (Driscoll et al. 1995; Mossman and Churg 1998; Oberdörster and Lehnert 1991; Saffiotti 1998; Tsuda et al. 1997). Chronic inflammation of the lungs is associated with the release of mediators such as cytokines (interleukin 1 (IL-1), tumour necrosis factor alpha (TNF-α)), chemokines (IL-8), macrophage inflammatory proteins (MIP), growth factors (PDGF, EGF, IGF, FGF) and arachidonic acid metabolites (prostaglandin E_2, leukotriene B_4). Studies in vivo found that glass wool, rock wool, slag wool and ceramic fibres can induce both the release of the tumour necrosis factor TNF-α and an increase in TGF-β and of the BAL parameters (influx of neutrophilic granulocytes (PMN, or polymorphonuclear neutrophils), release of lactate dehydrogenase and proteins) (Bermudez et al. 2003; Creutzenberg et al. 1997; IARC 2002; Morimoto et al. 1999, 2001; NTP 2010; Schürkes et al. 2004). Carbon nanotubes likewise induced chronic inflammation in exposed animals (Poland et al. 2008). After intratra-

cheal administration, MWCNTs caused a severe inflammatory response in the lungs of rats and mice (Kato et al. 2013; Porter et al. 2010).

Chronic inflammation plays an important role in the development of fibrosis. In studies with rodents, lung cancer almost always developed as a result of chronic inflammation and fibrosis of the lungs. However, a direct relationship between lung cancer and fibrosis has not yet been conclusively established (Kane et al. 1996). Nevertheless, a relationship between chronic inflammation, fibrosis and cancer is biologically plausible (NTP 2010).

2.3.5 Genotoxic effects

As mentioned in Section 2.3.2, both asbestos fibres and synthetic mineral fibres can cause DNA damage as a result of the release of ROS and RNS. If it cannot be reversed by the corresponding repair processes, it leads to permanent genetic mutations. Specifically, this refers to a spectrum of chemically modified DNA bases, DNA breaks, aneuploidy and DNA cross-links.

DNA changes induced by fibres can be differentiated, according to their activation, as follows:

- Direct mechanical interaction with the spindle apparatus or the cytoskeleton of the cell can occur, resulting in a loss of chromosomes or mis-segregation. This led to the generation of aneuploid cells, polyploid cells and binucleated and multinucleated cells after mammalian cells were incubated with long fibres in vitro (Dopp and Schiffmann 1998). However, these disruptive interactions have yet to be observed in vivo.
- Fibres that can activate oxygen or induce the enzyme iNO synthase as a result of their surface reactivity, that is, in particular because of the amount of catalytically active transition metals they contain, release the DNA-damaging intermediates ROS and RNS within target cells, for example in bronchial epithelial cells and mesothelial cells. In this way, a diverse range of modified DNA bases and deoxyribose fragments can also develop in the DNA strands (Cavallo et al. 2004; Elias et al. 2002; IARC 2002; Jaurand 1996; NTP 2010; WHO 2006). Such effects have also been observed in the case of asbestos fibres. However, synthetic mineral fibres are not as potent (Jaurand 1996; Mossman 2008; Nguea et al. 2008). In addition, the released ROS and RNS concomitantly inactivate tumour suppressor genes such as p53, which are involved in DNA repair (Ohshima et al. 2006; Pacher et al. 2007), while at the same time stimulating the DNA replication apparatus (Mossman et al. 2013), so that initially reparable DNA mutations are integrated into the genetic code. Increased cell proliferation is caused by the stimulation of cell membrane receptors by the above-named oxidants, including those of the epidermal growth factor or the tumour necrosis factor α (Mossman et al. 2013).
- However, the most important factor in the development of DNA mutations in bronchial and alveolar epithelial cells and in mesothelial cells is the inflammatory response triggered by synthetic fibres. This response is mainly caused by phagocytes, which accumulate at fibre deposition sites and release large amounts of ROS and RNS as they work to remove fibres or break them down in situ. This not only leads to a marked increase in the above-described oxidative DNA changes in the neighbouring cells ("target cells"), but new kinds of DNA damage occur as well, as

phagocytes greatly increase the levels of highly reactive NO intermediates. Nucleobases undergo nitrification and deamination and single-strand and DNA double-strand breaks are induced, which broaden the spectrum of damage overall and also have epigenetic consequences (Akuta et al. 2006; Sawa and Ohshima 2006). Thus, RNA-mediated deamination of 5-methylcytosine to thymine on the CpG islands in the promoter regions of the genes directly interferes in epigenetic gene regulation. It has been established that key proteins involved in DNA repair (Jones et al. 2009) and in the control of cell proliferation (Hussain et al. 2003) are attacked because of the chemical aggressiveness of peroxynitrite, which rapidly forms from $O_2^{\bullet-}$ and NO. This is expressed in the form of genetic and chromosomal mutations that contribute to carcinogenicity. However, findings from studies of synthetic fibres have yet to confirm this process in its entirety.

There are only a few in vivo studies available that investigated the genetic effects of synthetic mineral fibres (Kato et al. 2013; Osgood 1994; Schürkes et al. 2004; Topinka et al. 2006 a, b). These have established that there are qualitative and quantitative differences between the different synthetic mineral fibres. These are described in detail in the evaluation of the individual fibres.

It should be taken into account that most of the data for the genetic effects of synthetic mineral fibres have been obtained from in vitro studies. However, by today's standards, most of these investigations have methodological shortcomings and their findings have not been verified in vivo.

In summary, certain synthetic mineral fibres may have a genotoxic potential. However, the data currently available indicate that the direct genotoxicity of the fibres plays a rather subordinate role and is mainly dependent upon the oxidative status of the cells. Furthermore, the induced effects occur in parallel with the inflammatory processes.

2.3.6 Activation of signalling pathways

A large number of mechanistic studies have been published that investigated the effects of fibres and described in detail the signalling pathways using lung-typical cell lines. To date, these analyses have been carried out in phagocytes, bronchial epithelial cells and alveolar epithelial cells with asbestos and quartz (Albrecht et al. 2004; Castranova 2004; Fubini and Hubbard 2003; Mossman et al. 2006; Mossman and Glenn 2011; Ramos-Nino et al. 2003; see Figure 6). The signalling cascades that are activated in macrophages by synthetic mineral fibres are very similar to those that are activated by quartz. However, some of the data indicate that synthetic mineral fibres cause oxidative stress and activate proliferation also in epithelial cells of the lungs and mesothelial cells, provided that these fibres induce certain interactions; these are to be examined individually (Cullen et al. 1997). These interactions include: binding to the cell membrane of target cells, binding to specific cell receptors, the possibility of uptake into the cells by endocytosis (or phagocytosis) and the ability to trigger the generation of ROS and RNS inside the cells (Cardinali et al. 2006).

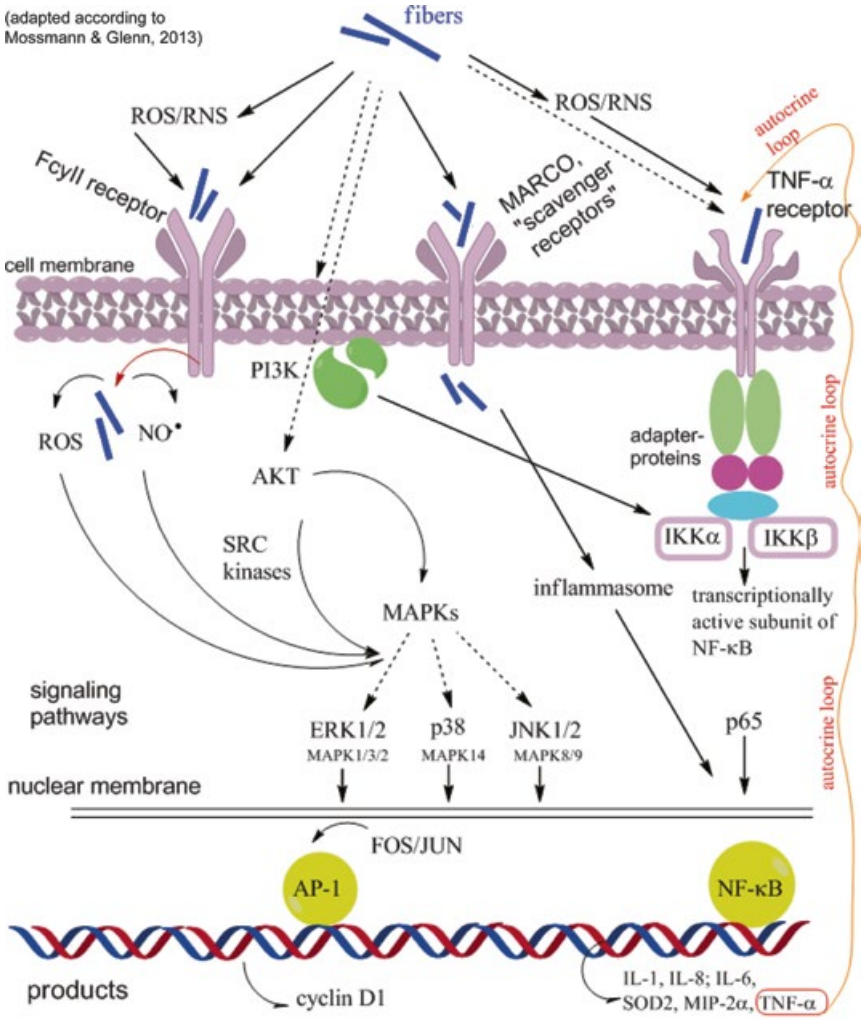

Figure 6 Mechanisms of action of biopersistent fibres in alveolar macrophages and lung epithelial cells (adapted according to Mossman and Glenn 2013).
English designations according to Human Genome Nomenclature and Universal Protein Resource. **MARCO**: macrophage receptor with collagenous structure; **SOD**: superoxide dismutase; **IKKα**: inhibitor of nuclear factor kappa-B kinase subunit alpha; **IKKβ**: inhibitor of nuclear factor kappa-B kinase subunit beta; **PI3K**: phosphatidylinositol 3-kinase; **ERK1/2**: **MAPK1/3/2**; **p38**: **MAPK14** (**JUN1/2**, stress-induced kinases); **AKT**: protein kinase B; **TNF-α**: tumour necrosis factor α; **AP-1**: activator protein, transcription factor; **SRC kinases**: non-receptor tyrosine kinases; **MIP-2α**: macrophage inflammatory protein 2α

A number of different signalling pathways are known to trigger an increase in the expression of genes that control growth-promoting processes such as the suppression of apoptosis, cell proliferation, inflammation and tumour promotion, etc.

These involve:

- the direct or TNF-α-mediated stimulation of the redox-sensitive transcription factor NF-κB, which induces the generation and release of cytokines (inflammation-promoting interleukins, chemokines and growth factors). This stimulation has been observed after exposure of epithelial A549 cells to MMVF10, RCF-1 and SiC fibres (Brown et al. 1999, 2002; Schins and Donaldson 2000). NF-κB can also be released by ROS/RNS-stimulated protein kinase B (AKT) or phosphorylated, that is, activated by phosphatidylinositol-3-kinase (see Figures 6 and 7).
- the multistep stimulation of stress kinases (ERK1/2; p38; JNK1/2), which in turn induce the expression of the target genes of cell proliferation (including the gene of cyclin D1, which initiates the cell cycle) via the activation of the respective transcription factors (JUN, FOS, FRA-1, e.g. AP-1). This signalling pathway can be activated in different ways by the interaction of fibrous dusts, either with the cell membrane itself or via an intermediate step involving cell surface receptors (for example integrins, EGF receptor). Protein kinase C can also be found at the beginning of a signalling pathway.
- the activation of protein kinase B (AKT). This suppresses apoptosis by inhibiting pro-apoptotic proteins (for example BAD, caspase 9), thus activating the anti-apoptotic system and at the same time stimulating DNA repair (Roos et al. 2016). This enables the proliferation of mutated cells that have been damaged by ROS/RNS.

2.3.7 Cytotoxicity and proliferation of target cells

Cell proliferation is initiated by tissue damage (cytotoxicity), among other things. After exposure by inhalation, synthetic mineral fibres induce cell proliferation in the terminal areas of the respiratory tract (Brown et al. 2002). It was demonstrated in vitro that the cytotoxicity of fibres is primarily dependent on their length (NTP 2010).

2.3.8 Epigenetic effects

ROS and chlorine radicals result from the stimulation of myeloperoxidase; these substitute OH groups or chlorine groups of cytosine bases in DNA at position 5. These have been identified as biological mechanisms that can in case of chronic inflammation lead to extensive epigenetic alterations (including the silencing of tumour suppressor genes) and thus to the development of cancer (Andujar et al. 2007; Valinluck and Sowers 2007; see Appendix).

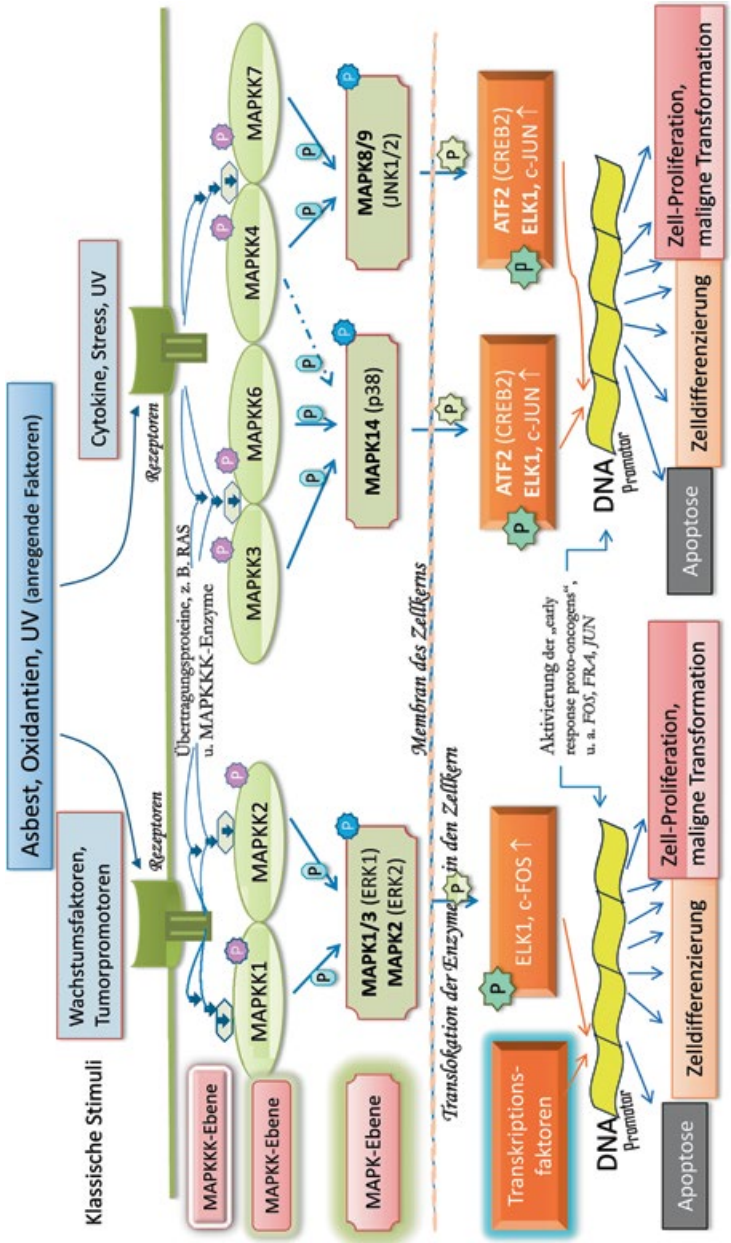

Figure 7 MAPK signal transduction pathways in mammalian cells induced by asbestos/oxidants (Ramos-Nino et al. 2002; modified), available in German only. **MAP signal transduction pathways in mammalian cells MAPKK1/2/3/4/6/7**: "dual specificity mitogen-activated protein kinase kinases 1/2/3/4/6/7/mitogen-activated protein kinase kinase". **MAPK1/2/3**: "mitogen-activated protein kinases 1/2/3 = ERK1/2" (ERK: "extracellular signal-regulated kinases"; activated primarily during growth-promoting processes, also those triggered by asbestos or H_2O_2). **MAPK14** (also MAP kinase p38 α–δ) and **MAPK8/9** (also JNK1/2: "c-JUN-*N*-terminal kinases 1/2/3") are activated by extracellular stress (e.g. UV light, hunger signals, osmotic changes, oxidative stress) and proinflammatory cytokines.

3 Toxicokinetics and Metabolism

3.1 Kinetics

3.1.1 Deposition

A large number of publications and reviews have described the principles of the deposition of inorganic fibres (amongst others Asgharian and Yu 1988; Bernstein et al. 2005; IARC 2002). An important factor that influences the deposition behaviour of fibres is the aerodynamic diameter, which is largely determined by the fibre diameter, but also the orientation of the fibre in the direction of flow. Additional factors are the physical properties of the fibres, anatomical and physiological parameters of the upper and lower respiratory tract including the pleura, and the fraction of deposited fibres, their clearance and translocation (Dai and Yu 1998).

Fibres are deposited on the surface of the respiratory tract by sedimentation, impaction, or interception; the efficiency of these processes correlates with fibre length. Exposure to particles generally increases the local concentration at anatomical structures, such as the bifurcations of the bronchi, which is why effects ranging from inflammation and tissue damage to tumours can be found primarily in these areas (documentation "General threshold limit value for dust (R fraction) (Biopersistent granular dusts)" 2012). However, the alveolar deposition of inhaled particles and fibres differs depending on the species and their anatomical features. This needs to be taken into consideration when extrapolating the findings from inhalation studies with animals to humans (IARC 2002). While it is quite unlikely that fibres with an aerodynamic diameter of > 3.5 μm will reach the alveolar region in rats and hamsters, the likelihood is greater in humans because of the larger human respiratory tract (Dai and Yu 1998). In inhalation studies with rats and mice, it was found that fibres were deposited primarily in the bifurcations of the first alveolar duct (Brody and Roe 1983; IARC 2002; Warheit et al. 1988). Other studies found that 50% of all fibres with an aerodynamic diameter of ≤ 0.1 μm reach the alveolar region of rats and are eliminated (Bernstein et al. 2005). For reasons of anatomy (size of the nose and shape of the nasal conchae) and physiology (nose/mouth breathers), quantitatively more and larger fibres are deposited in the alveolar region of human lungs than from those of rats. A comparison of fibre deposition in humans and hamsters found that also fibre length was an important factor (fibre length 3–5 μm: similar deposition fractions f_D; fibre length ≥ 15 μm: $f_D$human > $f_D$hamster) (Dai and Yu 1998).

The retained dose of inhaled particles is generally governed by the deposited dose minus the fraction eliminated by clearance (Bernstein et al. 2005); it is usually determined in animal experiments by measuring the fibres present in the lungs at different points of time. Due to impaired clearance persistent fibres can occur at very high local doses over long periods of time. Even without surface reactivity or the presence of toxic elements such as boron, these fibres can exhibit tumour-inducing potential, due to chronic inflammation.

Findings for nano fibres

In studies of silver nano wires (AgNW) that were 4 μm in length, pathogenic effects were observed after injection of the nano wires into the pleura of mice (Schinwald

et al. 2012 a). The deposition of CNTs into the lungs is dependent on their shape (curved, tangled or elongated) and whether they occur as individual fibres or agglomerate (Maynard et al. 2004; Shvedova et al. 2005). Like granular particles, it is likely that fibre agglomerates, depending on their aerodynamic diameter, are deposited in the lungs. As is the case with mineral fibres, individual elongated CNTs can reach as far as the lowest lung areas, penetrate the visceral pleura and enter the pleural cavity (Donaldson et al. 2006). Rigidity plays an important role in deposition, but also in clearance, translocation and finally also in the induction of effects (Nagai et al. 2011).

3.1.2 Clearance

Depending on the respective lung section, fibrous particles are removed by a range of clearance mechanisms (documentation "General threshold limit value for dust (R fraction) (Biopersistent granular dusts)" 2012; documentation "General Threshold Limit Value for Dust" 1999). All of these clearance processes affect the biopersistence of a fibre (IARC 2002). Biopersistence refers to the biodurability of a fibre over time and its clearance. Biodurability, in contrast, describes the resistance of a fibre to decomposition and disintegration and its solubility (NTP 2010). The following clearance mechanisms are involved in the elimination from the lungs:

Physiological processes:

- nasal and tracheobronchial mucociliary clearance
- removal by alveolar macrophages (limited for fibres that are longer than the macrophage diameter)
- interstitial translocation
- lymphatic clearance (limited for fibres longer than about 5 µm)

Physico-chemical processes:

- leaching of surface components from fibres
- dissolution of fibres
- breaking and thus shortening of fibres.

Particles and fibres are removed from the nasal and tracheobronchial region of the respiratory tract by mucociliary clearance (Lippmann et al. 1980). In the alveolar region, short fibres can be phagocytized and removed by macrophages or can penetrate to the interstitial space and be transported to the lymph nodes (Warheit and Gavett 1993).

Clearance is slower in the alveolar region than in the upper respiratory tract (IARC 2002; Pinkerton et al. 1993).

Fibre length is a limiting factor for macrophage-mediated clearance and for lymphatic clearance. Fibres that exceed the diameter of an alveolar macrophage cannot or can only be incompletely phagocytized (frustrated phagocytosis) (Dörger et al. 2000, 2001; Hamilton et al. 2009; Ji et al. 2012; Murphy et al. 2012, 2013; Ye et al. 1999). A study with mice found that peritoneal macrophages were no longer able to phagocytize elongated fibres (amosite, MWCNTs) of > 15 µm in length (Poland et al. 2008). These findings were confirmed by a study investigating the exposure

of mice to AgNW. Fibres > 14 μm in length are no longer phagocytized by alveolar macrophages; this induces an acute pulmonary inflammatory response (Schinwald and Donaldson 2012). A fibre length of > 14 μm can thus be considered the critical fibre length for the occurrence of frustrated phagocytosis in mice. Frustrated phagocytosis was observed in vitro in alveolar and peritoneal macrophages of rats and hamsters that were exposed to the mineral fibres MMVF10 (glass wool fibres, median fibre length ≈ 16 μm) and MMVF21 (rock wool fibres, median fibre length ≈ 19 μm) (Dörger et al. 2000, 2001). Macrophages differ in size from species to species. According to the literature, the diameter of alveolar macrophages in rats ranges from 10.5 to 13 μm and the diameter of alveolar macrophages in humans ranges from 14 to 21 μm (ATSDR 2004; Crapo et al. 1983; IARC 2002; Krombach et al. 1997; Lum et al. 1983; Sebring and Lehnert 1992; Stone et al. 1992).

Before long fibres can be cleared by macrophages, they first have to be broken down into smaller pieces so that they can be taken up by macrophages. In contrast, shorter fibres can be directly phagocytized by macrophages. For this reason, the elimination of persistent long fibres is much slower than that of shorter fibres. In contrast, soluble long fibres can break at sites of local corrosion and then be removed (Bernstein 2007 a, b; Hammad et al. 1988). A number of different studies have reported that, after a certain period of time, most of the fibres that remained were short biosoluble fibres.

It has been demonstrated that fibres and granular particles can penetrate to the pleural cavity and the peritoneal cavity. They are removed through the parietal lymphatic stomata (Donaldson et al. 2010; IARC 2009; Nagai and Toyokuni 2012). The severity of the inflammatory response was investigated after the injection of silver nano wires of varying lengths into the pleural cavity of mice (Schinwald et al. 2012 a). A length of 5 μm proved to be the critical limit for the removal of fibres through the stomata of the pleura and peritoneum (Schinwald et al. 2012 b; Schinwald and Donaldson 2012).

Physico-chemical processes can be reproduced and determined in in vitro systems to a certain extent. Transversal fragmentation (asbestos fibres break longitudinally) was observed in vitro (Bauer 1998). The physiological clearance processes that take place in vivo vary are species-dependent and cannot be reproduced in vitro (IARC 2002).

3.1.3 Translocation

A further important aspect is the possible interaction of the fibres with the lung epithelial cells, their subsequent penetration to the pulmonary interstitium and possible translocation to the pleura, the peritoneum or to more distant areas.

Neither active and passive transport nor the translocation pathways beginning in the lungs are fully understood. Incomplete or frustrated phagocytosis of persistent fibres can lead to interaction between these fibrous particles and alveolar cells and translocation to the pulmonary interstitium through the pleural membranes by means of the pressure gradient. In the pleura, fibres can induce disease through direct and indirect interaction with the pleural cells (Broaddus et al. 2011). In particular proteins, cells and liquids, also exogenous particles are removed from the pleural cavity through the lymphatic stomata, the openings between the mesothelial cells

of the parietal pleura (Broaddus et al. 2011). Lymphatic stomata are 2 to 12 µm in diameter (Donaldson et al. 2013; Li 1993; Li and Li 2003, 2004; Shinohara 1997). The stomata can be found primarily in the caudal area of the pleural/diaphragmatic region (= lymph drainage area). The condition of the lungs (for example, the inflammatory condition) has a marked effect on the lymphatic flow and thus also on the removal of the fibres from the pleura. Fibres that cannot pass through the stomata because of their size and rigidity accumulate at and block the openings to the lymphatic system.

It is assumed that biopersistent fibres are more likely than soluble fibres to penetrate to the interstitial space and to the pleural cavity, where they can induce an inflammatory response. In contrast, soluble fibres only remain in one place for a few days, which means that the respective dose in situ is much lower. This type of fibre, which, due to its composition, induces almost no toxic effects, is thus unlikely to have carcinogenic potential (Oberdörster 2002).

For this reason, fibre persistence and geometry are critical determinants of the pathogenic potential and thus also of carcinogenicity (Coin et al. 1992; Goodglick and Kane 1990; Lippmann 1990; McClellan and Hesterberg 1994; Stanton et al. 1981; WHO 1988).

As was demonstrated for silver and nickel nano wires and MWCNTs, the critical fibre length is 5 to 8 µm. In mice, fibres falling within this range were too long and inflexible to pass through the stomata into the lymph drainage system. Instead, such fibres adhere to the mesothelial lining (Schinwald et al. 2012 a). Fibres that pass through the stomata ultimately reach the regional lymph nodes along the lymphatic canals (Oberdörster 1988; IARC 2002). A study of the translocation behaviour of biopersistent fibres with nanoscale diameters demonstrated that, after a certain latency period, the fibres were enclosed by a protein sheath. In addition, fibre clusters formed, which severely impaired clearance by phagocytizing cells (Schinwald et al. 2012 a). A study with rats that were treated with MWCNTs by "intrapulmonary spraying" (a technique developed by the authors) found that these fibres were translocated from the lungs into the pleural cavity, where they induced an inflammatory response as well as hyperplastic visceral mesothelial proliferation (Xu et al. 2012). The fibres were localized primarily in the alveolar macrophages and in the focal granulomatous lesions in alveoli. They were also found in mediastinal lymph nodes, in liver sinusoid cells, in blood vessel walls, in brain cells and in renal tubular cells. However, only very few fibres directly penetrated through the visceral pleura and no fibres were detected in the parietal pleura. For this reason, the authors postulated that the lymphatic system was a possible translocation pathway.

In an inhalation study in mice treated with long, straight MWCNTs (length 100 nm–10 µm), fibres were observed in the macrophages, mesenchymal cells and in the collagen in the subpleural region already one day after inhalation (Donaldson et al. 2010; Ryman-Rasmussen et al. 2009).

3.2 Biopersistence

Biopersistence is a primary determinant of the fibrotic and carcinogenic effects of fibres.

Biosoluble fibres with a short retention half-life in the lungs induce almost no long-term effects (Oberdörster 2002). A significant step in assessing carcinogenic

potential could involve the derivation of a "threshold value" at which fibres are permanently retained in the lungs and pleura and are thus considered biopersistent. This would mean that biosoluble fibres that do not contain any toxicologically relevant components or cannot adsorb biological components could be classified as not carcinogenic (Oberdörster 2002). The chemical composition of the fibre surface and fibre geometry are key determinants of solubility (Bernstein et al. 2001, 2005; Bernstein 2007 a, b; Broaddus et al. 2011; Fubini 1997; Mossman and Glenn 2011).

Methods to investigate the biopersistence of glass wool fibres and rock wool fibres were set down in a directive of the European Union and have been included in the approval process since 1998 (EU 1997). Biopersistence is in general determined by exposure by inhalation or intratracheal instillation in rats. Biopersistence can be measured on the basis of 5-day or 90-day inhalation studies in rats that determined the half-lives of the fibres (> 20 μm or > 5 μm) in the lungs (Bernstein et al. 1996, 2005). For this test procedure it is important to use a fibre fraction that is inhalable by rats determined on the basis of complex aerodynamic processes. The mean geometric diameter of the fibre fraction corresponding to the WHO definition should be 0.6 μm and that for the fraction of fibres > 20 μm in length should be 0.8 μm. Data for fibre persistence that can be used by regulatory authorities for comparative assessments can be obtained only if conventions are in place with respect to the predefined fibre diameters.

Another established method in rats involves the intratracheal instillation of fibres in an aqueous suspension once or twice on successive days, followed by the determination of the amount of fibres with lengths exceeding 5 μm and 20 μm that are retained in the lungs (Muhle et al. 1994; Oberdörster 2002). In this case as well the fibres are subjected to an aerodynamic screening procedure prior to the beginning of testing to ensure that the diameter of the test fibres is equivalent to 0.6 μm or 0.8 μm. Likewise, it is important to investigate the morphology of the fibres in the lungs and in the pleura after different periods of time (2, 14, 30 and 90 days) and to determine whether any changes were induced that could be an indication of possible dissolution processes. The retention half-lives and biopersistence of different types of fibres were determined in a 5-day inhalation study in rats (Hesterberg et al. 1998) (Table 2). An overview of the findings pertaining to the biopersistence of about 60 different glass wool fibres and rock wool fibres was published by Bellmann et al. (2010).

In summary, the following rank order has been established for the degree of biopersistence: crocidolite > amosite > aluminium silicate fibres, special-purpose fibres (E-glass, glass type 475), rock wool (traditional variety) > glass wool > slag wool > rock wool (further-developed variety) (Davis et al. 1996; Hesterberg et al. 1998).

The findings show a positive correlation between increasing biopersistence (fibre length > 20 μm) and positive tumour findings in the lungs. A correlation between increasing biopersistence (medium fibre length 1.4–20 μm) and positive tumour findings was observed in studies with rats administered intraperitoneally with different types of fibres (aluminium silicate fibres, rock wool fibres, glass wool fibres, slag wool fibres, wollastonite, "Bayer" fibres (biosoluble mineral fibres that are specially manufactured for use as test fibres), C-glass, rock wool fibres type G) were given to rats by intraperitoneal injection (Adachi et al. 2001; Bernstein et al. 2001). It is possible to determine the carcinogenic potential of fibres and to rank their relative potency by giving defined fibre number concentrations in suspension to rats by in-

Table 2 Biopersistence and in vitro solubility of fibres of varying lengths (> 20 µm) in the lungs of rats (modified according to Hesterberg et al. 1998; Valic 2012)

Fibre		Biopersistence [d][a]	In vitro solubility rate [ng/(h cm²)][b]	Lung diseases	
				fibrosis	tumour
Asbestos	crocidolite	817	< 1	+	+
	amosite	418	< 1	+	+
	chrysotile	0.3–11.4[c]			
glass fibre	E-glass	79	9	+	+
aluminium silicate fibre	RCF1a	55	3	+	+
glass fibre	glass type 475	49	12	+	+/–[d]
mineral wool	rock wool MMVF12	67	20	+	–
glass fibre	JM 901	14.5	300	–	–
	Certain Teed B glass	9	100	–	–
mineral wool	slag wool MMVF11	9	400	–	–
	HT rock wool	6	59	–	–

[a] biopersistence determined as the weighted half-life of fibres in days, which is defined as the number of days required to eliminate 50% of the fibres from the lungs
[b] in vitro solubility rate at pH = 7.4 (Eastes and Hadley 1994)
[c] Bernstein et al. 2004, 2011; the published data diverges widely depending on the material
[d] positive findings in hamsters, but not in rats

traperitoneal injection. Unlike certain types of fibres, granular dusts do not induce mesotheliomas after intraperitoneal administration.

Inhalation studies with synthetic mineral fibres in rats revealed that the tumour-inducing potential increases with increasing biopersistence and the fibre fraction exceeding 20 µm (Bernstein et al. 2001). As discussed above, it takes considerably longer to eliminate long biodurable fibres than shorter fibres. As a result, the local dose of long fibres is expected to be much higher (dose being defined as the time integral of the local concentration) and it is assumed that the carcinogenic potential of one long fibre is greater than that of many short fibres, even if the short fibres, placed end to end, are of the same length as the long fibre (IARC 2002). However, shorter fibres can also have carcinogenic potential and fibre length is not an exclusion criterion (Dodson et al. 1990, 2003).

A certain level of biodegradation has been established for SWCNTs with surface carboxylic acid groups (oxidizing environment). This effect is not induced by CNTs with other surface functionalizations (Allen et al. 2008; Kagan et al. 2010; Liu et al. 2010). The surface modifications are thought to improve the biosolubility of CNTs (Donaldson et al. 2013). The degradation of four different MWCNTs without surface functionalization was investigated in vitro over a period of up to 24 weeks in

Gambles solution with a pH of 4.5 and the findings compared with those for amosite, chrysotile and a soluble glass fibre (Osmond-McLeod et al. 2011). Only one of the investigated MWCNTs yielded a 30% loss of mass after 3 weeks; no further loss of mass was recorded from weeks 3 to 24. The loss of mass was associated with a reduced fraction of long CNTs and a weaker inflammatory response after intraperitoneal injection in mice. The authors attributed 20% of the loss of mass to the method of measurement (filtering and weighing of the suspended samples). A loss of mass of about 25% was recorded also for crocidolite in this study.

Significance of kinetics for carcinogenicity in different species

In most cases, rats are used as models for in vivo studies. It should be borne in mind, however, that the half-life of biopersistent fibres might be longer than the lifespan of rats and hamsters, but shorter than the lifespan of humans (Berry 1999).

Furthermore, the different species react with varying degrees of sensitivity. According to the literature, the decreasing sensitivity of the species to fibres in the lungs is as follows: human > rat > hamster (Wardenbach et al. 2005; IARC 2002). However, hamsters are more sensitive than rats to the development of malignant pleural mesotheliomas (IARC 2002). In a medium-term inhalation study with exposure of hamsters to aluminium silicate fibres (RCF-1), an increased incidence of malignant pleural mesotheliomas was observed. There was a marked translocation of fibres, which accumulated primarily in the pleural cavity. In addition, greater proliferation of mesothelial cells was determined in hamsters than in rats (Gelzleichter et al. 1999).

Further differences between rats, hamsters and humans are found in the inhalability of fibres and differing translocation, which seems to be more pronounced in hamsters (Dai and Yu 1998; Gelzleichter et al. 1999). In addition, the visceral pleura is thinner in rodents and is composed primarily of a mesothelial layer and a submembrane, which rests directly on the peripheral alveoli. The submembrane is made up of thin submesothelial tissue and does not contain pleural blood vessels. Accordingly the translocation of fibres from the lungs to the pleura differs in humans and in rodents. However, the localization, accumulation and also the interactions of the fibres on the parietal pleura seem to be the same (Broaddus et al. 2011; Zocchi 2002).

4 Effects in Humans

A review found only slight evidence of an association between exposure to synthetic mineral fibres and the diagnosis of a mesothelioma (Boffetta et al. 2014). It should be noted that this review article does not meet today's quality requirements for methodically adequate systematic reviews; shortcomings include the absence of a systematic literature search in several electronic databases, a documented, independent double screening of the identified literature and a standardized assessment of the quality of the relevant studies. The authors of the review noted that, of a total of 6 mesothelioma cases found in the cohort studies included, 3 occurred after coexposure to asbestos. Three of 4 identified case–control studies found an increased risk of mesothelioma for workers who had been exposed to synthetic mineral fibres. However, the authors pointed out that the increased risk could have been caused by a residual effect of the confounding factor asbestos.

Likewise, earlier reviews (see IARC 2002, 2009) did not yield unequivocal evidence of an association between lung cancer and occupational exposure to synthetic mineral fibres. A review that included a meta-analysis found a statistically significant, but only slightly increased risk of lung cancer caused by exposure to synthetic mineral fibres (rock wool and glass wool) (Lipworth et al. 2009). The authors stated that no conclusion could be drawn concerning possible carcinogenic effects of synthetic mineral fibres because there was only a slight increase in risk, the risk was not increased for users, there was no dose–response relationship and there was probably some uncertainty with respect to the determination of exposure and the influence of the confounding factors smoking and asbestos exposure.

Previous negative findings from epidemiological studies are to be interpreted with caution for the following reasons: on the one hand, the level of exposure was considerably lower in these earlier studies than in the epidemiological studies of asbestos that yielded positive findings. Furthermore, the length of the observation periods of the earlier epidemiological studies of synthetic mineral fibres was too short in some cases, which means that it is too early to draw reliable conclusions about the effects that would be induced after the latency period, which is assumed to be comparable in length to that for asbestos exposure. Finally, any conclusions drawn after assessing synthetic mineral fibres as a collective group are of only limited value because of the disparity of effects caused by the different substances. The epidemiological findings for each of the substances are discussed in the documentations for the individual fibres.

5 Animal Experiments and in vitro Studies

Animal studies and in vitro studies and their findings are described and discussed in the documentations for the individual fibres.

6 Manifesto (MAK value/classification)

Fibre geometry has an effect on intrapulmonary deposition, translocation and clearance, and particularly on the uptake by phagocytizing cells. The shape and chemical composition of the fibres influence their degradation and their interaction with biomolecules and cell membranes and play an important role in fibre pathogenicity.

Even though the same range of biological defence mechanisms is available to the organism after exposure to both fibres and biopersistent granular particles, the greatest differences in the adverse effects induced by the two types of particles can be observed in the pleura. The dose–response relationship is greatly influenced by differences in deposition behaviour and elimination kinetics. Differences in fibre shape, surface reactivity and chemical composition also play a role and thus have consequences for the classification of fibres and the derivation of a limit value.

Carcinogenicity. Numerous epidemiological studies have shown that inhalative exposure to the fibrous dusts of asbestos and erionite induces tumours of the lungs, larynx, and ovaries in humans. Mesotheliomas of the pleura and, in several cases, mesotheliomas of the peritoneum and the pericardium were also found (IARC 2009;

NTP 2010). These effects have been confirmed by the findings from animal studies. In addition, certain synthetic fibres induce malignant lung tumours and mesotheliomas in rodents. However, these types of tumours have not been observed in humans to date. One possible reason for this could be that the required latency period has yet to be reached, another is that the level of exposure in the studies carried out up until this point was clearly lower than that in positive epidemiological studies of asbestos.

On the basis of the presently available data, it can be concluded that tumour development in the lungs and, on the basis of less reliable experimental data, also in the serous membranes (pleura, peritoneum, pericardium) is primarily a result of inflammatory processes. Macrophages, alveolar cells and mesothelial cells respond to impaired fibre clearance by releasing more proinflammatory cytokines and growth factors, which leads to chronic inflammation and cell proliferation. The same applies to the focal effects of fibres, which are accompanied by local inflammation and cell proliferation. In the process, indirect genotoxic effects are induced by the generation of radicals, reactive oxygen species and reactive nitrogen species as well as chlorine radicals. Recent studies suggest that the impairment of clearance also in the pleural cavity is to be considered a critical mechanism of action.

Other mechanisms depend on the type of fibre. These include: I) the generation of ROS and RNS by the fibre itself, II) uptake of the fibres into the target cells via endocytosis, leading to genetic and epigenetic changes caused by the intracellular release of ROS and RNS, and III) the stimulation of cell receptors and inflammasomes, which, in turn, trigger intracellular signalling pathways, thereby providing the impulse for cell proliferation and apoptosis resistance. The influence of these mechanisms cannot be estimated quantitatively on the basis of the data available at this time.

In principle, it is possible to derive and comply with a limit value for fibres to prevent chronic inflammation as well as proliferation-stimulating effects by apoptosis inhibition; this can be achieved amongst others on the basis of a sufficiently low biopersistence. Particular to poorly soluble fibres is that they cause the cleaning systems of the lungs and the serous membranes to reach the limits of their functionality not because of a volume overload of the macrophages, but because of their distinct geometry:

- poorly soluble, rigid fibres can get caught in the respiratory tract and locally penetrate into the cells
- long fibres are less easily removed via the mucociliary escalator or eliminated from the pleural cavity through the stomata
- long fibres that exceed the diameter of phagocytizing cells (including macrophages) can lead to frustrated phagocytosis

Fibres are classified in different categories for carcinogens depending on their properties and the data available. Classification in category 4 and a MAK value can be derived for those fibres that do not induce chronic inflammation in the lungs or in the pleura at the derived MAK value and do not elicit an overstimulation of proliferation in the case of apoptosis inhibition.

Therefore, in order to prevent adverse health effects, all inorganic fibrous dusts are considered to be potential carcinogens, unless findings from carcinogenicity studies would allow classification in a different category.

No classification is made if the fibres are sufficiently biosoluble in the lungs and in the pleural cavity.

MAK value. A limit value for fibres should ensure that fibre-induced inflammation in humans after long-term exposure, and also the chronic inflammatory and proliferative tissue changes in the lungs and pleura resulting from it do not take place. In addition to the development of tumours, the late sequelae of inflammatory changes include lung fibrosis, pleural fibrosis and the formation of pleural plaques.

Further end points such as **peak limitation, germ cell mutagenicity, pregnancy risk group and sensitization are evaluated and determined for the individual fibrous dusts.**

Appendix

In several places in Sections 2 and 3, comparisons were made with the relatively extensive data that have been collected for asbestos. However, it was expressly pointed out that asbestos is not the subject of the documentation "Fibrous Dusts, inorganic", but can be used as a point of reference in the discussion of the degree to which synthetic mineral fibres induce similar effects.

The following is a discussion of the genetic changes in asbestos-induced lung carcinomas and mesotheliomas, which occur often making a causal relationship likely, because they could also be observed after exposure to other fibrous dusts. Tables 3 and 4 and the explanatory text that follow supplement the data published in the IARC review from 2009. In addition, a relationship is established between fibre-induced inflammatory response and epigenetic changes.

Genetic changes in lung tumours and mesotheliomas in humans caused by exposure to asbestos fibres

A large number of genetic and epigenetic changes occur during what is often a lengthy latency period preceding the clinical diagnosis of cancer. These may include:

- activation of the genes for cell growth and regulation mechanisms
- mutations and amplifications of oncogenes
- inactivation of tumour-suppressor genes that can be characteristic for the specific histopathological types of these tumours (IARC 2009)

A range of methods has been applied to identify the genetic changes that occur in both types of tumour, lung tumours and mesotheliomas: comparative genomic hybridization, genome-wide transcriptome analysis, transfer of new sequence data into biochips for gene expression analyses, DNA cytometry and gene promoter analysis (Balsara et al. 1999; Björkqvist et al. 1997; Gordon et al. 2005; Krismann et al. 2002; Lopez-Rios et al. 2006; Murphy and Testa 1999; Singhal et al. 2003; Thomas et al. 2007).

Table 3 Genetic and epigenetic changes that occur with human bronchial carcinomas (modified according to IARC 2009)

Functional change	Gene target	Histological lung cancer type	
		small-cell	non-small-cell
stimulation of autocrine growth	growth factors and receptors	*GRP/GRP* receptor *SCF/KIT*	*TGFA/EGFR* *HGF/MET*
oncogene activation	*RAS* mutations	< 1%	15–20%
	MYC overexpression	15–30%	5–10%
inactivation of tumour suppressor genes	*TP53* mutation	~ 90%	~ 50%
	RB1 mutation	~ 90%	15–30%
	CDKN2A inactivation	0–10%	30–70%
	FHIT inactivation	~ 75%	50–75%
apoptosis resistance	*BCL2* expression	75–95%	10–35%
genetic instability	microsatellite instability	~ 35%	~ 22%

BCL2: B-cell CLL/lymphoma 2; *CDKN2A*: cyclin-dependent kinase inhibitor 2A; *EGFR*: epidermal growth factor receptor; *FHIT*: fragile histidine triad (dinucleosidetriphosphatase); *GRP*: gastrin-releasing peptide; *HGF*: hepatocyte growth factor; *KIT*: v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog; *MET*: met proto-oncogene; *MYC*: v-myc avian myelocytomatosis viral oncogene homolog; *RAS*: rat sarcoma viral oncogene homolog; *RB1*: RB transcriptional corepressor 1; *SCF*: stem cell factor; *TGFA*: transforming growth factor-α; *TP53*: tumour protein p53

Genetic changes occurring with lung cancer

Human lung cancer can be divided into two histological subtypes: the small-cell and the non-small-cell bronchial carcinoma, in which genetic changes occur with different frequencies (see Table 3).

Molecular changes that are induced by asbestos in non-small-cell carcinomas are the introduction of activating point mutations into the *KRAS* oncogene (Nelson et al. 1999), a loss of heterozygosity and point mutations in the *TP53* tumour suppressor gene of lung and larynx cancer (IARC 2009; Nymark et al. 2008).

Genetic changes in mesotheliomas

The above-mentioned analyses found that gene losses, gene inactivations, and also gene amplifications often occur in human mesotheliomas and affect the following chromosomal sites: 1p, 3p, 4p, 4q, 9p, 13q, 15q, 17p, 22q.

As the latency period for mesothelioma development can be 40 years or more (Bianchi and Bianchi 2007), it is to be assumed that the mannifold chromosomal changes occur gradually by selection according to the principle of growth advantage.

Other genetic and epigenetic changes that occur with malignant mesotheliomas are listed in Table 4. The gene and protein names and their acronyms listed in Tables 3 and 4 and in Figures 6 and 7 are in accordance with the standard nomenclature used

Table 4 Genetic and epigenetic changes that occur with human mesotheliomas (modified according to IARC 2009)

Functions	Gene target	Change
stimulation of autocrine growth	Growth factors and receptors	upregulation of *HGF/MET, EGFR, PDGF, IGF1*
tumour suppression	*CDKN2B, CDKN2A, CDKN2A/ARF* *NF2, BAP1* *RASSF1, GPC3*	inactivation; deletion *NF2* deletion; *NF2*, *BAP1* mutations hypermethylation
angiogenesis	*VEGF*	Upregulation
apoptosis	*AKT* *BCL2L1*	activation of upregulation

CDKN2A/ARF: cyclin-dependent kinase inhibitor 2A with alternate first exon containing an alternate open reading frame (ARF); AKT: v-akt murine thymoma viral oncogene homolog; BCL2L1: BCL2-like 1; BCL2: B-cell CLL/lymphoma 2; BAP1: BRCA1 associated protein (ubiquitin carboxy-terminal hydrolase); CDKN2A: cyclin-dependent kinase inhibitor 2A; CDKN2B: cyclin-dependent kinase inhibitor 2B (P15, inhibits CDK4); EGFR: epidermal growth factor receptor; HGF: hepatocyte growth factor; GPC3: glypican 3; IGF1: insulin-like growth factor 1; MET: met proto-oncogene, NF2: merlin; PDGF: platelet-derived growth factor; RASSF1: Ras association (RalGDS/AF-6) domain family member 1; VEGF: vascular endothelial growth factor A

worldwide in scientific literature. They were taken from the accepted, authoritative databases "Genome Nomenclature" (HUGO) and "Universal Protein Resource", which were influential in establishing the standard nomenclature.

Tables 3 and 4 show that driver mutations in asbestos-induced lung tumours and mesotheliomas are rare and furthermore varied. The results of recent, targeted next-generation sequencing of mesothelioma biopsies (Lo Iacono et al. 2014) reveal a complex picture of genetic changes with a large number of variations clustered primarily in two signalling pathways, the TP53/DNA repair pathway and the phosphatidylinositol-3-kinase/AKT-kinase pathway. Among other things, the latter controls glucose metabolism, cell proliferation processes, angiogenesis and apoptosis resistance. In addition, in more than 50% of the cases, whole-exome analyses of pleural mesotheliomas (Guo et al. 2015) yielded deactivating mutations in the control elements of two signalling pathways, such as MAP kinase (mitogen-activated protein kinase) and WNT cascade (wingless type signalling cascade). This affects in particular the genes *BAP1*, *NF2*, *CDKN2A* and *CUL*, whereby homozygous deletions predominate. A large number of other genes also are subject to mutations; however, the gene sites are random and individual, that is, each tumour has its own unique set of genetic alterations. Thus, the investigated mesotheliomas were all characterized by, on the one hand, few typical mutations and, on the other hand, also mutations that were found scattered across many gene sites. In agreement with earlier, isolated investigations, not only the mesotheliomas of different persons are affected by this genetic heterogeneity, but also the mesotheliomas of one and the same tumour carrier ("intra-tumour heterogeneity"; Carbone et al. 2015).

While mutations in the genes *TP53*, *EGFR, KRAS and CDKN2A* predominated in the non-small-cell lung tumours of patients exposed to asbestos, the *NF2* gene, which is almost regularly deactivated in mesotheliomas, was revealed to be unchanged (Andujar et al. 2013; Sekido et al. 1995). However, the genetic damage that occurs with lung tumours is quite frequently not only induced by asbestos, but also by other exogenous carcinogens, in particular tobacco smoke. This twofold burden results in homozygous deletions as also found in hypermethylated promoter regions. Point mutations are rarely observed (Andujar et al. 2010; Krauss et al. 2006).

On the mechanistic bridge between fibre-induced inflammation and epigenetic changes

Many sources have documented the genetic changes that are induced by inflammation-related reactive oxygen species and reactive nitrogen species. However, the pathway that leads from these reactive intermediates to DNA hypermethylation requires further elucidation. Numerous publications have found that inflammatory processes activate the enzymes NADPH oxidase and myeloperoxidase (Asahi et al. 2010; Babior 2004; Klebanoff 2005; Poulos 2014), which cooperate to form reactive intermediates (HOCl) from halogen anions (for example Cl^-). These, in turn, halogenate cytosine bases of DNA at position 5. Due to the physico-chemical properties of the chlorine ligand (sum of the van der Waals radius and the bond length; Valinluck and Sowers 2007; Valinluck et al. 2006), this substitution imitates a methyl group. Since 5-methylcytosine controls the epigenetic regulation of genes in the so-called CpG islands of the gene promoters, that is, mediates gene expression or deactivation, random chlorination inhibits any ordered gene regulation. The disruption can both convert proto-oncogenes into oncogenes and deactivate tumour suppressor genes ("gene silencing"). In addition, 5-methyl-CpG islands are hereditary DNA modifications and thus compel the methylation of the initially unmethylated DNA daughter strands during DNA replication. This means that prolonged inflammation triggers cumulative DNA changes over cell generations that promote malignant degeneration via their functional effects. Genetic changes such as mutations, deletions, insertions and epigenetic errors act in an interlocking manner during this process. The epigenetic errors are not limited to the sending of incorrect signals such as the pseudo-methylation of DNA, but also extend to the reading of these incorrect signals by recognition proteins and to the impairment of the mechanism of signal removal (Baylin and Jones 2011; Shen and Laird 2013).

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completed 21 July 2016