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Rome, 27–31 October 2008
Item 5 (e) of the provisional agenda*

Implementation of the Convention: consideration of a chemical for inclusion in Annex III of the Convention: chrysotile asbestos

Report of the World Health Organization workshop on mechanisms of fibre carcinogenesis and assessment of chrysotile asbestos substitutes

Note by the Secretariat

The annex to the present note contains the full report of the World Health Organization workshop on mechanisms of fibre carcinogenesis and assessment of chrysotile asbestos substitutes, which was held in Lyon, France, from 8 to 12 November 2005. A summary consensus report was made available at the third meeting of the Conference of the Parties. The full report is presented as received and has not been formally edited by the Secretariat.

* UNEP/FAO/RC/COP.4/1.

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WHO Workshop on Mechanisms of Fibre Carcinogenesis and Assessment of Chrysotile Asbestos Substitutes

8–12 November 2005

Lyon, France

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Secretariat for the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade

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Geneva, 25 March 2004

Subject: Chrysotile asbestos – assessment of alternatives.

Dear Dr Meredith

I refer to the request of the Intergovernmental Negotiation Committee at its tenth session to the World Health Organisation to conduct an assessment of alternatives to chrysotile asbestos. At this meeting, the WHO agreed that such an assessment would be able to be conducted, however requested that the fifth session of the Interim Chemical Review Committee for the Rotterdam Convention would consider the alternatives proposed by governments and develop a priority risk.. The Interim Chemicals Review Committee considered the alternatives proposed by governments, and developed a priority list for consideration by the WHO. They also developed a list of additional alternatives which were prioritised.

These alternatives are identified in the attached document, which is an extract from the report of the Interim Chemical Review Committee. I therefore invite the WHO to proceed with the agreed assessment of the proposed alternatives to chrysotile asbestos. If possible, it would be appreciated if an update on the progress of the assessment could be provided to the Intergovernmental Negotiating Committee at its eleventh session.

Please do not hesitate to contact us should you have any questions in regard to this letter.

Yours sincerely,

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Annex I

Report of the contact group on chrysotile

1. The contact group considered the list of substitutes for chrysotile asbestos proposed by Governments for assessment by the World Health Organization (WHO). WHO indicated that it welcomed the guidance provided by the group on important alternatives used by Governments.

2. The list was prioritized initially on the basis of the number of Governments which had nominated the substances. Information on which substances had previously been assessed in environmental health criteria reports by IPCS was also considered. Where possible, the group's knowledge of important uses was also considered.

3. The first group of substances are listed on a priority basis, in the order in which the contact group would like them to be considered by WHO. The second group of substances, which were proposed by only one country, had undergone no previous assessment by WHO and could be considered if resources allowed.

Group 1: Substances identified and prioritized for assessment by WHO

Aramid and para-aramid fibres
Fibrous glass (glass fibres, glass wool)
Carbon/graphite
Ceramic fibres
Wollastonite
Cellulose fibres
Mineral wool (rock wool, slag wool)
Polyvinyl alcohol (PVA) fibres
Polypropylene fibres
Polyvinyl chloride (PVC) fibres
Attapulgite
Polyethylene fibres

Group 2: Substances identified as alternatives to chrysotile, to be assessed if resources allow

Aluminium silicates, basic magnesium sulphate whisker, erionite, ductile iron, mica, phosphate, polyacryl nitryl, polytetrafluoroethylene, potassium titanate whisker, semi-metallics, silicon carbide whisker, steel fibres

World Health Organization

Workshop on Mechanisms of Fibre Carcinogenesis and Assessment of Chrysotile Asbestos Substitutes

Lyon, 8-12 November 2005

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[4] The Fraunhofer Institute is doing contract research for many companies that produce glass wool, rock wool, or ceramic fibres.

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List of acronyms

8-OH-dG	8-hydroxydeoxyguanosine
8-oxo-dG	8-oxo-7,8-dihydro-2′-deoxyguanosine
AM	alveolar macrophages
ATP	adenosine triphosphate
ATPase	adenosine triphosphatase
BAL	bronchoalveolar lavage
BET	Brunauer, Emmett, and Teller
BHT	biological half-time
BrdU	5-bromo-2-deoxyuridine
CFE	colony forming efficiency
CI	confidence interval
CMS	calcium magnesium silicate
CMZS	calcium magnesium zirconium silicate
DMPO-OH	5,5-dimethyl-1-pyrroline *N*-oxide–hydroxyl radical
DNA	deoxyribonucleic acid
DNase	deoxyribonuclease
EC_{50}	median effective concentration
ELISA	enzyme-linked immunosorbent assay
EPR	electron paramagnetic resonance
FISH	fluorescence in situ hybridization
GLP	Good Laboratory Practice
GMD	geometric mean diameter
GML	geometric mean length
GSD	geometric standard deviation
GSH	reduced glutathione
GSSG	oxidized glutathione
h-AM	alveolar macrophages from humans
h-RBC	human red blood cells
HTE	hamster tracheal epithelial
h-TII	type II pneumocytes from humans
IARC	International Agency for Research on Cancer
IgA	immunoglobulin A
IL-1	interleukin-1
INC	Intergovernmental Negotiating Committee
k_{dis}	dissolution rate constant
L	lung
LDH	lactate dehydrogenase
LN	lymph nodes
MMVF	man-made vitreous fibres
mRNA	messenger ribonucleic acid
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
mvt	?
NIOSH	National Institute of Occupational Safety and Health
ODC	ornithine decarboxylase
P	particles
PAN	polyacrylonitrile
PCOM	phase-contrast optical microscopy
PL	pleural lavage

PLM	polarized light microscopy
PMN	polymorphonuclear leukocyte
PVA	polyvinyl alcohol
PVC	polyvinyl chloride
PVC-E	PVC emulsion process
PVC-S	PVC suspension process
r-AM	alveolar macrophages from rats
RCC	Research and Consulting Company
RCF	refractory ceramic fibre
RFP	respirable-sized, fibre-shaped particulates
RNA	ribonucleic acid
RNase	ribonuclease
RNS	reactive nitrogen species
ROS	reactive oxygen species
RSNO	nitrosothiol
r-TII	type II pneumocytes from rats
SD	standard deviation
SDH	succinate dehydrogenase
SEM	scanning electron microscopy
SHE	Syrian hamster embryo
SIR	standardized incidence ratio
SMR	standardized mortality ratio
SOD	superoxide dismutase
TEM	transmission electron microscopy
TGF	transforming growth factor
TII	type II pneumocytes
TLC	thin-layer chromatography
TNF-α	tumour necrosis factor alpha
TPA	12-*O*-tetradecanoyl-phorbol-13-acetate
UDS	unscheduled DNA synthesis
UK	United Kingdom
USA	United States of America
VC	vinyl chloride
VCM	vinyl chloride monomer
WHO	World Health Organization

Part 1

Invited Papers on Mechanisms of Fibre Carcinogenesis

These papers are currently being finalized. They were prepared as background for the workshop and are the opinions of the individual authors and not the workshop.

Part 2

Workshop Report

This is an unedited advance version of the meeting report, being made available for the purpose of providing information to the Conference of the Parties to the Rotterdam Convention.

2.1 General principles for the evaluation of chrysotile asbestos substitutes

2.1.1 Key considerations for evaluating epidemiological evidence

Epidemiological studies on fibres have a clear advantage over toxicological studies in that they involve studies of humans. They also have the advantage that they study the effects of exposure in the real world where the effects of these exposures may be mitigated or enhanced by other factors. For example, there is a well recognized synergistic interaction between smoking and asbestos (Vainio & Boffetta, 1994).

Despite these obvious advantages, the presence or absence of evidence of a risk from epidemiological studies does not always override contrary findings from toxicological studies, for a variety of reasons. It is frequently difficult to establish causality based solely on epidemiology because of the non-experimental nature of these studies. The lack of randomization in epidemiological studies makes it difficult to fully exclude the possibility that confounding or other forms of bias may be responsible for a positive association found in an epidemiological study. Negative—or what should perhaps be more appropriately referred to as "non-positive"—studies rarely, if ever, provide sufficient evidence for rejecting causality. The lack of positive evidence might be explained by limitations of the study design. For example, for recently introduced fibres, studies conducted to evaluate the risk of lung cancer would clearly have inadequate follow-up time and would thus be uninformative. Non-positive studies may still be highly informative, particularly when they are studies that have adequate statistical power to detect an effect, have good characterization of exposures and are unlikely to have substantial potential for confounding or other biases. At the very least, a well conducted study with a non-positive finding may be used to estimate an upper bound on what risk might be plausible.

Because of these considerations, the interpretation of either positive or non-positive epidemiological findings needs to be carefully considered in light of the strengths or weaknesses of the study design. Following is a discussion of some of the key issues that need to be considered, particularly with regard to the interpretation of the findings from epidemiological studies of fibres.

2.1.1.1 Statistical power

Although well designed epidemiological studies provide the most relevant information for the assessment of risks in human populations, the ability of these studies to detect an effect when it is present, generally referred to as the power of the study, depends on the size of the effect, the size of the study and, for effects with long latent periods, the duration of the study. The lessons learnt from occupational cohort studies of asbestos workers have led to much lower fibre exposures in the workplace, so that one would expect to detect cancer risks only in large cohorts followed up for a sufficiently long period of time. This fact should be kept in mind in the interpretation of occupational cohort studies that do not report increased risks of lung cancer or mesothelioma, which is primarily the case for the fibres that are reviewed in this document. These studies should be interpreted not as being negative, but rather as being non-positive. The statistical power of these studies to detect an effect is a critical consideration when interpreting the degree to which the lack of evidence for a carcinogenic effect is

persuasive or not. A key example of this concern is the study of refractory ceramic fibre (RCF) workers, which is a relatively small cohort (942 workers) that is young (mean age = 51 years) and has been followed for a relatively short period of time (mean length of follow-up of 21 years) (LeMasters, 2003). The degree to which this study had adequate power to detect an excess of lung cancer or mesothelioma is a concern, which is discussed in section 2.2.12.1.

2.1.1.2 Adequacy of exposure assessment

To estimate exposure, usually environmental dust samples are collected. In work environments, personal monitoring is accomplished by mounting a sampler on a worker in the breathing zone. Misclassification of exposure in epidemiological studies of fibres, as in all epidemiological studies, is potentially a serious problem. Errors resulting from misclassification of exposures generally may bias exposure–response relationships.

Samples are analysed for the number of fibres by different counting methods, such as phase-contrast optical microscopy (PCOM), scanning electron microscopy (SEM), polarized light microscopy (PLM) and transmission electron microscopy (TEM). For counting purposes, a fibre is defined as a particle >5 μm in length with a length to diameter ratio of at least 3:1 and a diameter of <3 μm; this type of fibre is often referred as a World Health Organization (WHO) fibre (WHO, 1996). According to National Institute of Occupational Safety and Health (NIOSH) counting rule "B", a fibre is defined as any particle >5 μm in length with a length to diameter ratio of at least 5:1 and a diameter of <3 μm. The difference in count between the WHO fibre and the NIOSH counting rule "B" fibre can be significant (Breysse et al., 1999). The exposure estimates for individual studies may differ based on which counting rule is used to count fibres. Thus, it is important to consider these differences in exposure estimates when comparing studies.

All counting methods (i.e. TEM, PCOM, SEM, PLM) are unable to distinguish individual organic fibres, so these are usually categorized as "other organic" fibres, which include polyvinyl alcohol (PVA) fibres, cellulose fibres and other textile fibres (clothing, bed sheets, blankets, pillows).

In occupational settings, exposures are mainly estimated and can be supported by industrial hygiene data. The exposure estimations can be based on personal samples, area samples, job matrices or processes involved. Most of the time the samples are dust samples, and respirable fibres are part of the samples. Thus, when epidemiological studies are evaluated, the confounding effect of other exposures, such as dust particles and other chemicals, should be taken into account. Most fibre studies indicate that exposures of installers and removal workers are much higher than exposures of workers involved in manufacturing and processing the same fibres. Many times direct information about exposure may not be available, but biomarkers of exposure, if present, are a helpful indication of relevant exposure (e.g. pleural plaques in asbestos exposure).

In evaluating carcinogenicity of any substance in humans, exposure information/assessment is usually the weakest link, and fibres are no exception to this rule.

2.1.1.3 Confounding and multiple exposures

Confounding in epidemiological studies is a bias that occurs when the exposed and the non-exposed groups have different disease risks even if they were not exposed to fibres (Pierce &

Greenland, 2004). More generally, confounding may occur when the groups to be compared (exposed and non-exposed) are not completely comparable. Confounding in studies of fibre-induced lung cancer could be the result of various factors, such as cigarette smoking, socioeconomic status or exposure to fibres, particles or other substances not being studied. Confounding can bias an effect in either direction, thus leading to the observation of higher or lower risk. Confounding can be controlled in the study design, analysis or both by means of randomization, restriction and matching in the design phase and by stratification or statistical adjustment in the analysis plan.

Mixed exposures can be taken to mean that a person is exposed to the fibre in question as well as to other fibres, particles or substances that produce the same effect. It may be that the situation of mixed exposures cannot be treated as a classical problem of confounding because exposure to fibres of concern may be highly correlated with exposures to other fibres, particles and substances (Cordier & Stewart, 2004). Stratified analysis and multivariate modelling may not be effective in adverse situations with high colinearity among exposures.

2.1.2 Significance of short- and long-term studies in experimental animals in the assessment of the potential hazards of fibres to human health

End-points in humans that are covered are the long-term effects, notably cancer and fibrosis. Effects such as skin irritation and byssinosis, nylon flock worker's lung, are not considered.

The major route of exposure in humans is inhalation. Oral exposure falls outside the scope of this document.

2.1.2.1 Short-term animal experiments

Methodology

Fibre dimensions of the aerosol should approach as closely as possible the fibre dimensions encountered in human exposures. However, as respirability, which is mainly dependent on fibre diameter, differs between rats and humans, special attention should be given to fibre length. It makes little sense to increase artificially the fibre length of the aerosol in animal inhalation studies if humans are exposed mainly to much shorter fibres.

Lung burden/number and fibre dimensions should be determined at appropriate time points during and after exposure. This indicates whether exposure concentrations have been adequately chosen, and it gives an impression of the behaviour (disappearance, breakage) of the fibres in the lung.

The adequacy of the study design and performance should be verified by the use of a positive control.

In studies on fibrosis and inflammation, it is important that a recovery group be part of the design.

Effects

Many end-points have been measured. These yield supportive information vis-à-vis toxic effects (lung weight, bronchoalveolar lavage [BAL]), but the discussion here is limited to those that may have a relation to carcinogenicity or fibrosis.

Fibrosis (as demonstrated histopathologically in animal experiments) can be determined if exposure is sufficiently long (90 days), especially when a recovery group is examined. Fibrosis has not been demonstrated to be a prerequisite to carcinogenesis.

Epithelial cell proliferation is a repair mechanism after tissue injury, but proliferation of target cells for carcinogenesis above background levels can be regarded as an essential step in carcinogenesis, as it increases the probability of mutated cell populations. Of special concern is increased cell proliferation if it persists after the end of exposure.

A relationship of epithelial cell proliferation with cancer in experimental animals could be demonstrated for amosite, crocidolite, MMVF21, and special-purpose glass fibres E and 475.

After intraperitoneal injection of fibres, proliferative changes might also be studied in peritoneal cells, which are the origin of mesothelioma. The available data show that proliferation of mesothelial cells is greater with fibrous than with granular dust.

Analysis of BAL indicates the extent of lung **inflammation**. It is supportive information on fibrosis, especially related to effects in silica exposure, but less clear-cut in the case of fibres.

Histopathology of the lung can indicate a carcinogenic potential (especially metaplasia).

2.1.2.2 Long-term animal experiments (inhalation)

The primary aim is to detect fibrosis and/or carcinogenic response.

The predominant tumour type in rats after exposure to certain fibres has been lung cancer, but after exposure to amphibole asbestos, a low incidence of mesothelioma has also been reported in rats.

Methodology

This concerns both inhalation and intraperitoneal studies in rats.

Fibre dimensions of the aerosol should approach as closely as possible the fibre dimensions encountered in human exposures. However, as respirability, which is mainly dependent on fibre diameter, differs between rats and humans, special attention should be given to fibre length. It makes little sense to increase artificially the fibre length of the aerosol in animal inhalation studies if humans are exposed mainly to much shorter fibres.

Lung burden/number and fibre dimensions should be determined at appropriate time points during and after exposure. This indicates whether exposure concentrations have been adequately chosen, and it gives an impression of the behaviours (disappearance, breakage) of the fibres in the lung.

The adequacy of the study design and performance should be verified by the use of a positive control.

Exposure concentrations of long-term inhalation studies in rats should be high enough to make it possible to exclude with some confidence a carcinogenic potency similar to the potency of amphibole asbestos. Taking into account the existing chronic inhalation data with amphibole asbestos, not respirable, fibre concentration should at least amount to 1000 fibres/ml with length >5 μm, 200 fibres/ml with length >200 μm and 100 fibres/ml with length >20 μm. These concentrations represent the lowest aerosol concentrations of amphibole asbestos in rat inhalation studies that resulted in increased incidence in lung tumours. If such a minimum exposure concentration is not applied in a chronic rat inhalation study, the validity of non-positive results is highly questionable.

If for various reasons it is not possible to achieve the desired concentrations (e.g. high mass concentration because fibres are rather thick, improved sizing was not successful), exposure of experimental animals by intratracheal or intraperitoneal injection should be performed.

(Information on the carcinogenicity in experimental animals is derived from studies on natural mineral fibres and on man-made vitreous fibres [MMVF], whereas much less is known on the carcinogenicity of organic fibres and also of carbon fibres.)

Sensitivity

All fibres that have been shown to cause cancer in epidemiological studies in humans have also been shown to be carcinogenic in animals by inhalation or intraperitoneal injection.

From studies with asbestos, it is apparent that the sensitivity of the rat to fibre-induced lung tumours in inhalation studies is clearly lower than that of humans. This holds true when the effect is related to exposure concentrations and lung burdens. Differences in size of organs and lifespan might be responsible for this observation. The question remains open as to whether this sensitivity difference remains if individual rat or human lung cells are taken as a basis for comparison.

Specificity

Several fibres that have been shown to be carcinogenic in animals have not been demonstrated to be carcinogenic in epidemiological studies. This can be attributed either to the lack of epidemiological investigations or to the various factors that result in non-positive studies (for further details, see epidemiology subsections in section 2.2).

Target organ concordance

The main target organs for fibre carcinogenicity in humans are the lung, pleura and peritoneum, whereas in experimental animals, notably rats, cancer is mostly seen in the lung (in inhalation exposure), mesothelioma being rare. Thus, there is a partial target organ non-concordance between humans and rats. Target organ non-concordance is more rule than exception in chemical carcinogenesis (IPCS, 2005).

Intraperitoneal injection

Many more fibres have been tested using intraperitoneal injection compared with inhalation exposure.

Compared with rat inhalation studies, carcinogenicity testing of fibres by intraperitoneal injection represents a sensitive assay. With this test system, it is easily possible to detect the carcinogenic effects of fibres whose potency is more than 2 orders lower than the potency of crocidolite.

In studies using intraperitoneal injection, there is no indication that granular particles induce tumours themselves; thus, the confounding by granular materials is excluded in these studies. The mechanisms of carcinogenicity in pulmonary cells after inhalation exposure may not be identical to those in peritoneal cells.

The rank order of potency (per fibre) of those fibres that have been tested by both routes is in general similar.

Because of the low sensitivity of the rat inhalation model for lung tumours and mesothelioma, the intraperitoneal route of administration is a valuable addition to the test battery for fibre carcinogenicity.

2.1.3 Use of in vitro short-term tests in assessing fibre carcinogenicity

Short-term tests are frequently applied in toxicology, aiming to provide some information on the genotoxic and transforming potential of chemicals. Since fibres are distinctly different from chemical mutagens and/or carcinogens, this raises the question as to whether and under which circumstances short-term assays, particularly cell culture studies, are suited to predict fibre genotoxicity and whether the outcome of short-term studies is relevant for fibre-induced carcinogenicity.

Carcinogenesis is a multistep process involving the generation of deoxyribonucleic acid (DNA) damage by genotoxic agents. The cellular response system involves DNA repair systems, cell cycle arrest and, in the case of heavily damaged cells, apoptosis. Nevertheless, some lesions will lead to mutations; growth advantage of initiated cells and increased chromosomal instability are further steps towards tumour formation.

As best investigated for asbestos, after deposition in the different compartments of the respiratory system, fibres can act on early and later steps of tumour formation; current knowledge on mechanisms involved in asbestos-induced carcinogenicity can be summarized as follows. Deposited fibres in the lung may produce reactive oxygen species (ROS) and lipid peroxidation products as a result of their own surface reactivity. More ROS and reactive nitrogen species (RNS) are generated because of phagocytosis of fibres by epithelial cells and macrophages; in addition, fibres may interact with the spindle apparatus in epithelial cells and therefore induce chromosome missegregation and chromosomal damage. Further steps include the recruitment of inflammatory cells and the release of mitogenic stimuli. ROS and RNS produce DNA and chromosomal damage, enhanced mutation rates and increased cell proliferation. Therefore, exposure to fibres may affect the initiating as well as the promotion step in neoplastic transformation.

Identification of fibre genotoxicity in experimental systems can be achieved via cell-free in vitro assays, in vitro tests with cultured cells and in vivo studies, usually in mice or rats. This section gives an overview of the various assays that are available and the suitable/optimal test conditions that should be fulfilled. In addition, for the in vitro tests with cultured cells and for the short-term in vivo assays, an assessment is made of their predictive value with respect to fibre carcinogenicity.

2.1.3.1 In vitro test systems

Following the sequence of events in the carcinogenic process, a number of end-points can be identified for which an effect after exposure to fibres can be measured in genotoxicity assays.

DNA damage can be determined as DNA strand breaks and oxidative DNA base modifications (e.g. 8-oxo-7,8-dihydro-2′-deoxyguanosine, or 8-oxo-dG) in isolated DNA or in cultured cells. Commonly used tests are, for example, single-cell gel electrophoresis (comet assay) and alkaline DNA unwinding in the presence or absence of DNA repair enzymes to measure DNA breakage; and high-performance liquid chromatography in combination with electrochemical detection to measure 8-oxo-dG in plasmid or cellular DNA.

Cytogenetic effects can be measured in cultured cells in the form of various types of chromosome-type and chromatid-type aberrations in metaphase cells and as anaphase or telophase abnormalities. By use of chromosome-specific fluorescent probes in combination with fluorescence in situ hybridization (FISH), information can be obtained on the involvement of individual chromosomes in the cytogenetic effects. Micronucleus formation can be determined in dividing cells. The micronuclei can be further characterized by use of centromere-specific probes, which allow discrimination between clastogenic and aneugenic origins of the micronuclei.

Mutations can be measured in cell culture systems that are suitable for the detection of large deletions.

Cell proliferation and **cell transformation** can be tested in cultured mammalian cells that are suitable for assessment of these end-points (e.g. Syrian hamster embryo [SHE] cells, NIH 3T3 cells, BALB/c-3T3 cells).

2.1.3.2 Suitable test conditions and requirements

With regard to the design of in vitro tests to determine fibre genotoxicity, the following criteria were identified:

- **Cell type:** Mammalian cells should be used that are capable of phagocytosis (i.e. of engulfing the fibre). Suitable cell lines and cell types are V79 hamster cells, Chinese hamster ovary cells, human A549 lung cells, primary and transformed mesothelial cells, lung cells, etc. Lymphocytes are less well suited, since they do not appear to be capable of phagocytosis. For mutagenicity studies, a cell type that allows detection of large deletions is required.

- **Fibre characteristics:** In a properly described assay, the concentration of fibres should be given in μg fibre/cm^2 and, additionally, in number of fibres per microgram. Information on fibre dimensions, chemical composition, relative surface area, size distribution and behaviour in physiological media (e.g. sedimentation versus floating in the culture medium) should also be provided.

- **Dose range:** An appropriate fibre dose range should be included in the assay in order to establish a dose–response relationship.

- **Phagocytosis and cytotoxicity:** These two end-points should be assessed to verify that the fibres have entered into the cells and that cell survival is sufficiently high for the test result to be reliable.

- **Incubation time:** The duration of exposure to the fibres should be sufficient to allow phagocytosis and subsequent cellular reactions to occur before the assay is performed. For measurement of cytogenetic effects, a total incubation time that allows 1.5 cell cycles is required before analysis.

- **Controls:** An appropriate positive control (usually asbestos) should be included in the study.

2.1.3.3 Predictive value of short-term cell culture tests for fibre carcinogenicity

When applying appropriate test conditions as outlined above, short-term tests pick up effects related to the initiation of the carcinogenic process, including genotoxicity related to surface properties of the fibres, phagocytosis as well as genetic changes during mitosis.

The significance of the respective effects is closely related to the genetic alterations identified. For example, DNA lesions can still be repaired, but mutations are fixed genetic alterations; chromosomal instabilities play an important role in tumour development.

Many genotoxic effects are related to the formation of ROS, which are also generated during normal cell metabolism, giving rise to measurable background levels of oxidative DNA base modifications; nevertheless, in the case of (continuous) imbalance between prooxidative and antioxidative effects, including DNA repair systems, this may result in increased mutation frequencies and genetic instability.

In contrast to primary genotoxic effects, effects related to biopersistence of fibres (e.g. continuous “frustrated phagocytosis”) will not be picked up. Secondary genotoxicity arising from ROS and RNS and mitogen release by macrophages and inflammatory cells are not detected either. Therefore, negative results indicate a lack of primary genotoxicity, but do not exclude effects on later steps of carcinogenesis. Furthermore, if the fibres under investigation do not sediment but float in the medium, short-term genotoxicity tests on adherent cells in culture are likely to be non-informative, and negative results do not indicate the absence of genotoxicity.

Altogether, as outlined above, fibres may act in principle on all steps in tumour development; however, of these interactions, the in vitro genotoxicity tests are mainly indicative of genotoxic effects involved in the first steps of tumour initiation.

2.1.3.4 Predictive value of short-term in vivo animal studies for fibre carcinogenicity

Test systems include conventional animals but also new animal models such as transgenic/knockout animals. Relevant end-points are DNA damage, mutations, chromosomal aberrations, micronuclei, homologous recombination, loss of heterozygosity and mitogenesis.

With respect to genotoxicity, short-term in vivo assays may provide an important bridge between short-term cell culture assays and genetic alterations related to carcinogenicity. In addition to primary genotoxic effects, secondary effects, such as mitogenic stimuli, macrophage and inflammatory cell interactions, may be picked up as well. These models may also provide information on mechanistic aspects, such as the impact of DNA repair on fibre-induced carcinogenesis.

2.1.4 Physicochemical properties and biopersistence

2.1.4.1 Chemical composition: origin, purity, variability of components, crystallinity and surface area

Fibres proposed as chrysotile asbestos substitutes comprise a chemically and structurally heterogeneous group that can be divided into several sets, depending on their nature (organic or inorganic) and origin (natural or artificial). Their chemical composition is a key factor influencing structure and physicochemical properties, such as surface area, surface reactivity and solubility. Attention should be paid not only to the chemical composition of the fibres, including their major and trace elements, but also to the contaminants or accompanying elements, including their speciation.

The chemical composition of the fibres should be stated very precisely, particularly in the presence of elements that may speciate as toxic moieties in vivo (e.g. arsenic, chromium) or that may be associated with carcinogenic effects (e.g. iron in asbestos). These elements as well as other substances can be incorporated during industrial production of synthetic fibres or may be naturally present in the mineral fibres. In the case of natural fibres, accessory minerals or contaminants may be associated with carcinogenic potency (e.g. quartz).

Crystallinity influences surface reactivity and solubility, because crystalline materials are more stable (less soluble) than their amorphous equivalent. Fibrous minerals frequently exhibit a narrow range of chemical composition. However, potentially hazardous trace elements can be present.

Silica-based synthetic vitreous fibres are amorphous fibres of variable composition. In addition to silica, which represents their major component, other elements may be present in variable proportions: aluminium, magnesium, calcium, sodium, potassium, iron, etc. The overall chemical composition is a key parameter controlling solubility and surface reactivity, as well as other physical or mechanical properties considered below.

A number of classification schemes have been proposed, based upon such factors as the origin of the material (pure oxides [glass wool] versus minerals [rock wool]), the ratio between silica and alumina, or use. The great variability in composition complicates a coherent classification. Because of the overlap of their constituents and physicochemical

properties, silica-based vitreous fibres were treated as a class. Recommendations were made about specific members of this class, depending upon their compositions, physical properties and biopersistence.

The specific surface of fibre types may differ, so that at equal exposure/dose expressed in mass, different surface areas may be exposed. Surface-driven effects have to be compared on a per unit surface basis.

Relevance to carcinogenicity

The release of carcinogenic elements that speciate and the presence at the fibre surface of elements known to impart carcinogenicity (e.g. iron in asbestos) are aspects of chemical composition that are relevant to carcinogenicity.

2.1.4.2 Bulk material, exposure and material for biological testing

For many fibrous materials, the bulk material cannot be used for experimental inhalation studies, for various reasons. For example, the bulk fibres may be too thick or too long to be respirable by the test species. Therefore, milling of the fibres may be necessary. Also, fibres may contain binders that prevent aerosol generation. For organic fibres, special treatments, such as removal of lignin from cellulose fibres, may be necessary to produce respirable fibres.

Relevance to carcinogenicity

Different preparations may yield different outcomes in in vivo tests.

2.1.4.3 Fibre dimension and deposition

The quantity of fibres retained in the lung is the net result of the amount deposited minus the amount cleared. The fraction of inhaled particles deposited in the respiratory tract can be calculated from their aerodynamic diameter, defined as the geometric diameter of a sphere of unit density that has the same terminal settling particle as the particle in question. In general, inhaled particles with a large aerodynamic diameter are predominantly deposited in the upper respiratory tract, and inhaled particles with a small aerodynamic diameter tend to be deposited in the pulmonary (alveolar) region. For fibres, the aerodynamic diameter (D_A) has been estimated by the formula (Stöber, 1972):

$$D_A = 1.3 \times p^{1/2} \times d^{5/6} \times l^{1/6}$$

where p is density, d is diameter and l is length. Accordingly, the fibre diameter determines aerodynamic diameter, and hence its regional deposition in the respiratory tract, to a significantly greater extent than fibre length.

Additional modelling of alveolar fibre deposition indicates that an increasing aspect ratio (length/diameter) is followed by a decreased deposition fraction. As deposition of inhaled fibres also depends on anatomical and physiological parameters of a given species, this modelling shows that there is a significant interspecies difference in alveolar deposition; more and larger fibres deposit in the respiratory tract of humans than in rats or hamsters. For rats and hamsters, there is hardly any alveolar deposition when the aerodynamic diameter of

the fibres exceeds 3.5 μm and the aspect ratio is >10. In humans, considerable alveolar deposition occurs even when the aerodynamic diameter of the fibres approaches 5 μm. Taking into account additional physiological parameters (minute volume, surface area of the alveolar epithelium), it was estimated that for a given inhaled concentration of fibres with an aerodynamic diameter of 2 μm and an aspect ratio of 20, the dose per unit surface area of the lung is about 10-fold larger in humans than in rats (IARC, 2002).

Injection and inhalation studies have shown that the longer, thinner and more durable fibres show a greater carcinogenic potency (Pott & Friedrichs, 1972; Stanton & Wrench, 1972; Davis et al., 1986; Roller et al., 1996). However, the inhalation studies are far less convincing in this respect (Wardenbach et al., 2005). On the basis of human data, mesothelioma was concluded to be correlated to the number of fibres >5 μm in length and <0.1 μm in diameter, and lung cancer to the number of fibres >10 μm in length and >0.15 μm in diameter (Lippmann, 1988). These fibre dimensions were also used to assess the health risks of MMVF (Lippmann, 1990). However, based on tissue analysis of mesothelioma cases and taking methodological aspects into account (e.g. detection limit), others argue that asbestos fibres of all length contribute to pathological responses (Suzuki & Yuen, 2001, 2002; Dodson et al., 2003).

Relevance to carcinogenicity

One can assume that there exists a continuous variation in the carcinogenic potency of respirable fibre, which increases with length.

2.1.4.4 Solubility and surface reactivity

The role of solubility and surface reactivity in fibre carcinogenesis is largely discussed by Kane et al. (1996) and in recent reviews (IARC, 2002; ILSI, 2005). The key points are summarized below.

Differences in solubility in water, in biological fluids and in vivo

Solubility is regulated not only by the solvent but also by the solutes, which may adsorb and/or selectively remove some fibre components, favouring fibre degradation. Thus, solubility in body fluids and in vivo is often much greater than the chemical solubility in water.

Chemical composition and solubility

Ionic components of the fibre (e.g. alkaline and alkaline earth ions in vitreous fibres) favour solubility, whereas other components (e.g. aluminium) decrease solubility. Stable polymeric carbon chains yield less soluble materials, but in some cases they may undergo enzymatic cleavage in vivo.

Surface reactivity in relation to free radical generation, surface hydrophilicity/hydrophobicity, fibre coating and protein adsorption

Surface composition regulates fibre uptake, protein adsorption, free radical generation and release of metallic ions, which are implicated in pathogenic processes (Fubini et al., 1998).

The presence of iron at the fibre surface plays a crucial role in most of the above processes (Hardy & Aust, 1995; Kamp & Weitzman, 1999).

Iron may be part of the chemical composition of the fibres, as it is in most amphibole asbestos and in slag and rock wools, may be present as a substitute of similar ions (Mg^{2+} in chrysotile asbestos) or may be present as an impurity acquired from the environment or endogenously (Fubini & Otero-Arean, 1999). Not all iron species are equally active (Gulumian et al., 1999; Fenoglio et al., 2001), whereas powdered iron oxides are fully inactive (Fubini & Mollo, 1995).

Fibre-derived free radicals are generated at iron centres—even in trace amounts—at the fibre surface. ROS and RNS are also produced following fibre cell contact in vitro and in vivo. Fibre-generated and cell-generated reactive species may subsequently react.

The degree of surface hydrophilicity/hydrophobicity determines wettability and floating and regulates cell surface adhesion, protein denaturation and uptake of endogenous molecules, which influence toxicity to cells and inflammatory response (Brown et al., 1992; Tomatis et al., 2002b). The bioactivity of an inhaled fibre is also influenced by the adsorption of proteins and lipids from the fluid lining of the respiratory tract (Wallace & Keane, ????). Wettability and floating may influence the results of in vitro studies.

Relevance to carcinogenicity

Free radical generation favours DNA damage and mutations. Surface properties are a determining factor in the inflammatory response.

2.1.4.5 Clearance and biopersistence

As discussed above, the dose of an inhaled fibre retained in the respiratory tract at any time is equivalent to the deposited dose minus the amount cleared. Several physiological clearance mechanisms contribute to a fibre's elimination from the lung, which include (ILSI, 2005):

- removal from the nose and tracheobronchial region by the mucociliary escalator;
- phagocytosis by alveolar macrophages in the alveolar region;
- interstitial translocation of deposited fibres, including translocation to the pleural sites;
- clearance via lymphatic channels once fibres have reached the interstitium.

The biopersistency of a fibre is a measure of its ability to remain in the lungs despite these clearance mechanisms, which are mediated by specific fibre properties, such as leachability, dissolution and breakage. A number of test systems, including inhalation and intratracheal instillation assays conducted according to well defined protocols and direct measurements of fibre dissolution by recording mass loss/unit surface area/unit time in simulated lung fluids, have been used to measure a fibre's tendency to biopersist.

In an analysis of a number of inhalation tests conducted on silica-based synthetic vitreous fibres, Moolgavkar et al. (2001) showed that the carcinogenic potential of a fibre is directly related to its weighted half-life, defined as the linear combination of short- and long-term clearance half-lives. Specifically, it was shown that the unit risk for lung cancer is approximately a linear function of the weighted half-life, when the weighted half-life is short

enough that the fibre burden in the lung reaches equilibrium in a time span shorter than the lifespan of the test species.

Relevance to carcinogenicity

Biopersistence of the fibre increases tissue burden and therefore may increase any toxicity the fibre might possess. For synthetic vitreous fibres, there is evidence in animals that the potential for carcinogenicity increases with biopersistence. This has not been demonstrated for other fibres.

2.2 Assessment of selected chrysotile asbestos substitutes

2.2.1 Aramid and *para*-aramid (Table 2.1)

2.2.1.1 Epidemiological studies

No data are available.

2.2.1.2 Animal studies

The data presented refer to *para*-aramid exclusively; no data were found on *meta*-aramid.

Carcinogenicity by inhalation

Development of keratinizing cysts in the lungs of rats after long-term inhalation exposure was not considered as an indication of carcinogenicity in the IARC (1997) evaluations. The study was terminated after 24 months.

Carcinogenicity by intraperitoneal injection

No data are available.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

At 280 fibres/cm^3 after 2 weeks, fibrotic thickening was observed, which nearly recovered after 4–6 months (Lee, 1983, cited in Warheit, 2001). In the 2-year study, there was also fibrosis after exposure to >25 fibres/cm^3. In the Bellman et al. (2000) study, fibrosis and hyperplastic lesions were observed in the medium- and high-dose (200 and 800 fibres/ml) groups directly after the end of the exposure.

Proliferation

No in vivo studies are available.

Confidence in database

The working group notes that there is an unpublished carcinogenicity study that was not included, as it is not publicly available.

2.2.1.3 Mechanistic data

Clearance and changes in situ of the deposited fibres/biopersistence

Inhalation toxicity studies with rats comparing the biopersistence of *p*-aramid fibres (respirable-sized) with asbestos fibres have demonstrated that the aramid fibres were less biopersistent than chrysotile asbestos. The longer *p*-aramid fibres were shortened in the lungs of rats, whereas longer chrysotile asbestos fibres were preferentially retained (Warheit et al., 1996). Fibre clearance studies of Warheit et al. (1992, 1997, 2002) demonstrated a transient increase in the numbers of retained fibres at 1 week postexposure, with a rapid clearance of

fibres thereafter. The transient increase in the number of fibres could be due to transverse cleaving of the fibres, since the average length of retained fibres continued to decrease over time.

Also, other groups have demonstrated a rapid clearance of the longest fibrils during the first month following subchronic inhalation exposures, and this was shown to be associated with a corresponding increase in the number of shorter fibrils (Kelly et al., 1993; Searl, 1997; Bellmann et al., 2000).

Warheit et al. (1999) reported on a study in which rats were exposed for 5 days to aerosols of *p*-aramid fibrils (900–1344 fibres/cm^3, 9–11 mg/m^3). The number of *p*-aramid fibrils per lung showed a transient increase at 1 week postexposure and then a rapid decrease thereafter; lung clearance half-life was approximately 30 days. During the 6 months in the lung, the *p*-aramid fibril mean length progressively decreased from 12.5 μm to 7.5 μm.

In another study mentioned by Warheit et al. (1999), rats were exposed to *p*-aramid fibrils by inhalation for 2 years. The initial mean dimensions of the inhaled fibres were 12 μm length and <0.3 μm diameter. After a 2-year exposure at 2.5, 25 and 100 fibrils/cm^3, mean lengths of lung-retained fibrils approached 4 μm. The time required for the fibrils to be reduced to <5 μm in the lung was markedly less at lower exposure concentrations.

Warheit et al. (2005) showed in in vivo as well as in vitro experiments that the mean lengths of *p*-aramid respirable-sized, fibre-shaped particulates (RFP) incubated with human or rat BAL fluids were substantially decreased compared with those incubated in phosphate-buffered saline. The authors concluded from their investigations that *p*-aramid RFP are likely to be biodegradable in the lungs of humans.

Modes of action of fibres in pulmonary/pleural carcinogenesis

Cystic keratinizing lung lesions produced following exposure to *p*-aramid RFP were observed by several authors (reviewed by Warheit, 1995). However, according to Frame et al. (1997), these lesions are not relevant for human risk assessment of pulmonary cancer.

Genotoxicity

p-Aramid RFP were tested for induction of chromosomal aberrations in cultured human peripheral blood lymphocytes (fibre concentrations: 6–400 μg/ml; exposure time: 19 h). No significant increase of chromosomal aberrations, polyploidy or endoreduplication was found in the exposed cells (Warheit et al., 2001).

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

The proliferative capacity of aramid (Kevlar) as well as colony forming efficiency (CFE) and ornithine decarboxylase (ODC) activity were measured by Marsh et al. (1994) in hamster tracheal epithelial (HTE) cells and in rat lung fibroblasts (RL90). In HTE cells, aramid

caused a statistically significant increase in [^{3}H]thymidine incorporation and CFE and produced a dose-dependent induction of ODC enzyme activity. Proliferative effects by aramid were not observed in RL90 fibroblasts.

Chronic inflammation, release of cytokines, growth factors, reactive species

In a study by Warheit et al. (1996), rats were exposed for 2 weeks to aerosols of *p*-aramid fibrils (750 fibres/cm^3). Two weeks' exposure to these fibres produced transient pulmonary inflammatory and cell labelling responses in terminal bronchiolar and subpleural regions.

Short-term animal tests/carcinogenicity studies

In a study by Lee et al. (1988), four groups of 100 male and 100 female rats were exposed to ultrafine Kevlar fibrils at concentrations of 0, 2.5, 25 and 100 fibrils/cm^3 for 6 h/day, 5 days/week, for 2 years. One group was exposed to 400 fibrils/ml for 1 year and allowed to recover for 1 year. At 25 fibrils/cm^3, the lungs showed a dust cell response, slight Type II pneumocyte hyperplasia, alveolar bronchiolarization and a negligible amount of collagenized fibrosis in the alveolar duct region. At 100 fibrils/cm^3, the same pulmonary responses were seen as at 25 fibrils/cm^3. In addition, cystic keratinizing squamous cell carcinoma was found in four female rats, but not in male rats. The lung tumours were derived from metaplastic squamous cells in areas of alveolar bronchiolarization. At 400 fibrils/cm^3 following 1 year of recovery, the lung dust content, average fibre length and pulmonary lesions were markedly reduced, but slight centriacinar emphysema and minimal collagenized fibrosis were found in the alveolar duct region. The lung tumours were a unique type of experimentally induced tumours in the rats and have not been seen as spontaneous tumours in humans or experimental animals. Therefore, the relevance of this type of lung tumour to the human situation is minimal.

Warheit et al. (1999) reported on a study in which rats were exposed to *p*-aramid RFP for 2 weeks, then maintained for a period of postexposure recovery. Rats exposed to lower levels (up to 26 fibres/cm^3) showed only a macrophage response, and those exposed to higher levels ($\geq$280 fibres/cm^3) developed granulomatous lesions at the alveolar duct bifurcations with fibrotic thickening. By 6 months postexposure, the granulomatous lesions were nearly recovered, and the fibrotic lesions were much reduced. During lung residence, the fibres fragmented and decreased in size at a rapid rate.

Summary on the determinants of carcinogenic potency

In 1995, the German Research Association (DFG) organized a workshop to reach an agreement on the criteria for the classification of cystic lesions. The cystic keratinizing lung lesions produced following exposure to *p*-aramid and many other dusts appear to be unique to the rat. The general opinion was that these lesions are probably not relevant for human risk assessment of pulmonary cancer.

In 1997, *p*-aramid fibrils (RFP) were evaluated by the International Agency for Research on Cancer (IARC) and it was judged that they "cannot be classified as to their carcinogenicity to humans" (Group 3). This decision was based on no data in humans and inadequate evidence in animals to show either the presence or absence of a carcinogenic effect.

Warheit et al. (2005) concluded from their studies that inhaled *p*-aramid RFP are likely to be biodegradable in the lungs of humans.

References

[References being compiled]

2.2.1.4 Physicochemical properties and biopersistence

p-Aramid releases fibrils with diameter <1 μm. Aramid fibres are approximately 10 μm in length. The fibres are respirable. Aramid is crystalline. Its physicochemical properties are similar to those of known carcinogenic fibres.

The lung clearance half-life of *p*-aramid was 30 days after a 5-day inhalation exposure. In other experiments, clearance was 60–170 days after subchronic exposure to 50–800 fibrils, respectively, and 6–9 weeks.

2.2.2 Attapulgite (Table 2.2)

2.2.2.1 Epidemiological studies

A single cohort study of palygorskite (attapulgite) miners and millers was available. It showed small excesses of mortality from lung cancer and stomach cancer, but no indications of any exposure–response for either cancer.

2.2.2.2 Animal studies

Carcinogenicity by inhalation

In one inhalation study in rats with attapulgite from Leicester, United Kingdom, in which about 20% of the fibres were longer than 6 μm, bronchoalveolar hyperplasia and a few benign and malignant alveolar tumours and mesotheliomas were observed.

In several studies involving exposure of rats by inhalation to short fibres (<0.5% longer than or equal to 5 μm), no increase in the incidence of tumours was observed.

Carcinogenicity by intraperitoneal/intrapleural injection

The attapulgite sample from Leicester described above also induced a high incidence of pleural mesotheliomas in rats after intrapleural administration.

One sample of attapulgite, in which 0.5% of the fibres were longer than 6 μm, produced a significant increase of pleural mesotheliomas after intrapleural administration.

Intraperitoneal injection of attapulgite with 30% of the fibres longer than 5 μm, and of another attapulgite, in which 3% of the fibres were longer than 5 μm, induced malignant abdominal tumours in rats.

In several studies involving exposure of rats by intrapleural or intraperitoneal administration to short fibres (<0.5% longer than or equal to 5 μm), no increase in the incidence of tumours was observed.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

In the long-term inhalation study of Wagner et al. (1987), the fibrosis score was 3.2 after exposure to <2 μm attapulgite but 4.0 after exposure to attapulgite with fibres longer than 6 μm.

Proliferation

No data are available.

2.2.2.3 Mechanistic data

There is limited evidence for induction of ROS. There are insufficient data to assess genotoxicity.

2.2.2.4 Physicochemical properties and biopersistence

Short fibres are <5 μm in length, and long fibres are >5 μm in length. The fibre diameter is 0.5 μm. Mineral impurities (including iron) have been measured. There are ambiguous data on free radical generation, which depends on the presence of iron in the solution and may not be a property of the fibre.

2.2.3 Carbon fibres (Table 2.3)

2.2.3.1 Epidemiological studies

No data are available.

2.2.3.2 Animal studies

Carcinogenicity by inhalation

No valid study is available (in the only existing study, no tumours were observed, but the fibres were not respirable).

Carcinogenicity by intraperitoneal injection

No data are available.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

In two 16-week studies with rats, no fibrosis was detected at an exposure concentration of 20 mg/m^3 (fibre number not stated; fibre diameters were 3.5 and 7 μm).

Proliferation

No data are available.

2.2.3.3 Mechanistic data

No data are available.

2.2.3.4 Physicochemical properties and biopersistence

Carbon fibres are <200 μm in length (median 40 μm), with a diameter between 3 and 8 μm. They are amorphous.

2.2.4 Cellulose fibres (Table 2.4)

2.2.4.1 Epidemiological studies

A cohort mortality study of 30 157 male workers who had worked for at least 1 year in 14 pulp and paper mill plants in British Columbia, Canada (1950–1992), was conducted by Band et al. (1997). Standardized mortality ratios (SMRs) were computed using Canadian population mortality rates as a comparison. Of 4047 deaths, 1052 were due to cancer. Statistically significant excesses were found for lung cancer (SMR = 1.32) as well as for cancer of other sites, such as pancreas, brain, liver, larynx, skin melanoma, Hodgkin disease and multiple myeloma in sulfite process workers and kidney cancer in the kraft process workers who had >15 years of employment. For workers who worked in both processes, a statistically significant excess was observed for non-Hodgkin lymphoma only. Increased pleural cancer mortality was observed in the total cohort (n = 8, SMR = 2.65, 90% confidence interval [CI] = 1.32–4.78). Of eight pleural cancers, five (SMR = 3.61, 90% CI = 1.42–7.58) were in workers who had ≥15 years of employment. Duration of employment was used as a surrogate for exposure. No specific information for exposure to fibres, other chemicals or smoking was provided.

Band et al. (2001) conducted a cancer incidence study in male workers from 14 pulp and paper mill plants in British Columbia (1950–1992). Cancer incidence of the cohort was compared with that of the Canadian population to compute standardized incidence ratios (SIRs). A total of 1756 cases were observed in 28 278 workers who had at least 1 year of employment. Because the person-year distribution and the case distribution was similar for the workers who had >15 years and <15 years from first employment, a cut-off of 15 years for latency was selected. Significantly increased SIRs were observed for skin melanoma and prostate cancer among kraft workers; for cancers of the liver, pancreas and lung (SIR = 1.32) among sulfite workers; and for skin melanoma and prostate cancer among workers who worked in both kraft and sulfite processes. Ten pleural cancers were reported (SIR = 2.05) in this population. The SIR for mesothelioma was statistically significant when all the workers were considered together, but not in individual process groups. The authors assert that the mesotheliomas were probably due to past asbestos exposure. Length of employment was used as a surrogate for the exposure. It is unclear to what extent the cohort was exposed to cellulose fibres, and there were confounding exposures to other chemicals as well. No information on smoking was available.

In a cohort of 63 025 long-term pulp and paper mill workers from 51 plants who had 10 or more years of employment, Matanoski et al. (1998) found that both the total mortality and the cancer mortality were low. Investigators collected employment data for the current workers and created a job dictionary with job titles, tasks, work areas and subwork areas based on steps in the paper-making process. The six work areas included in assigning the workers and for analysis were pulping, papermaking, finishing, power/recovery, mill-wide services and others. The mill processes and changes that occurred over the years were described. SMRs were computed using United States population mortality rates and 20 county-specific rates where the mills were located. Excess lung cancer SMR (1.35, 95% CI = 1.04–1.75) was observed among employees who worked in the kraft pulping process. The adjustments were done for age and calendar time. No specific information on exposures to fibres or smoking was available. The authors acknowledged the presence of several other chemicals.

In a Danish paper mill study of 14 362 workers employed any time during the follow-up period from 1943 to 1993, no excess lung cancer incidence was observed (Rix et al., 1998). Danish population cancer incidence rates were used to calculate the SIRs. The statistically significantly increased SIRs were observed for Hodgkin disease, pharyngeal cancer and soft tissue sarcomas. No specific information about exposure to fibres was provided.

2.2.4.2 Animal studies

There exists a marked heterogeneity within cellulose and cellulose-containing materials (e.g. bleached kraft cellulose, thermomechanical pulp, microcrystalline cellulose, cellulose for thin-layer chromatography [TLC], insulation cellulose).

Carcinogenicity by inhalation

No studies are available.

Carcinogenicity by intraperitoneal injection

The respirable fraction of cellulose fibre was collected from an aerosol of thermomechanically treated wood pulp, which contained, besides cellulose, other components, such as lignin. Twice as many cellulose fibres (24%) were long (>15 μm) as compared with the crocidolite fibres; the total dose of fibres was 116 mg for cellulose and 1.8 mg for crocidolite. The total doses injected intraperitoneally into rats were 10^6, 10^7, 10^8 and 10^9 WHO fibres. Crocidolite (10^8 or 10^9 WHO fibres) was used as the "positive" control, but no tumours were observed in this group. Nine of 50 animals of the 10^9 WHO fibres cellulose group had malignant tumours not derived from mesothelium (sarcomas); two animals in the low dose groups (one in each) developed mesotheliomas (Cullen et al., 2002).

Cullen et al. (2002) concluded that a high dose of cellulose fibres is capable of producing tumours when injected into the abdominal cavity of rats. However, the non-positive findings after crocidolite injection at high doses, as well as the unusual tumour type and their early appearance after cellulose administration, render it questionable as to whether the tumours observed were caused by the general mechanisms of fibre carcinogenesis.

In a long-term study in rats, where softwood kraft pulp (length:diameter 3:1; 1.5×10^4 fibres) was injected intraperitoneally into rats, no tumours were observed. The dose was considerably lower than that in the Cullen et al. (2002) study (Rosenbruch et al., 1992).

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

Cellulose after a single intratracheal dose (15 mg per animal) brought about fibrosing granulomatous alveobronchiolitis and an increase of immunoglobulin A (IgA) production in the BAL (Tatrai et al., 1996). Fibrosing alveolitis showed moderate progression as a function of time. At this dose level, almost any material would induce fibrosis.

Proliferation

No studies are available.

Confidence in database

Considering the variability of the cellulose products, the database to judge cellulose fibres as an entity is weak.

2.2.4.3 Mechanistic data

Deposition of inhaled fibres in the different parts of the respiratory tract

In a study of lung biopsies, Pauly et al. (1998) stated that cellulose fibres were detected in 99 of 114 specimens from exposed persons in the United States of America (USA). The fibres were identified by light microscopy, but the frequency and deposition of inhaled fibres were not reported.

Clearance and changes in situ of the deposited fibres/biopersistence

Muhle et al. (1997) showed that cellulose fibres are more persistent than chrysotile in the rat, but high doses were used, and the clearance was probably impaired (Harrison et al., 1999). In a newer study by Warheit et al. (2005), cellulose RFP was used as a biopersistent reference control fibre to *p*-aramid. The cellulose fibres were not measurably shortened compared with *p*-aramid.

Modes of action of fibres in pulmonary/pleural carcinogenesis

According to Cullen et al. (2002), cellulose fibres are durable and have the potential to persist within the lung. These authors injected high-purity cellulose fibres into the abdominal cavity of rats. In the highest-dose cellulose group, multiple large nodules (granulomas) and widespread adhesions (bands of new tissue connecting organs to each other and to the abdominal wall) were present in all animals. Granulomas were not observed in the 10^9 fibres crocidolite group. More than 80% of animals in the 10^8 and 10^9 fibres crocidolite asbestos groups had mesotheliomas. In contrast, there were only two animals in the cellulose groups with mesothelioma tumours. However, nine (18%) animals in the 10^9 fibres cellulose group had malignant tumours that, in contrast to the usual pattern of mesothelioma development following treatment with mineral fibres in rats, showed no obvious involvement of mesothelial tissues, were not associated with blood-stained ascites fluid and were thus classified as sarcomas. Cullen et al. (2002) concluded that a high dose of cellulose fibres is capable of producing tumours when injected into the abdominal cavity of rats.

Genotoxicity

No data are available.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

No data are available.

Chronic inflammation, release of cytokines, growth factors, reactive species

Moore et al. (2001) investigated the inflammatory response to cellulose acetate fibre materials by the respiratory burst of neutrophils and found positive effects. Apoptosis was increased 12% with exposure to the more aged fibres versus 2% with the new fibres. The authors suggested that neutrophils are activated by cellulose acetate and display an altered response to more aged fibres.

Cellulose fibres were found to be toxic to mouse macrophages in vitro (Godelaine & Beaufay, 1989a,b). Samples derived from pure cellulose fibre, including one where the particle size was in the respirable range, caused the release of more LDH than did similar doses of chrysotile or crocidolite. Cellulose fibre was also found to stimulate the release of inflammogenic materials from macrophages. Cells treated with cellulose and those treated with asbestos released similar amounts of plasminogen activator and IL-1. Cellulose proved more powerful than asbestos in stimulating the release of the inflammogenic agents prostaglandin PGE2 and leucotrine LTC4. Control dust, including glass and rock wool preparations, produced a much lower response.

Short-term animal tests

The inflammatory effects of respirable cellulose fibres were studied by Cullen et al. (2000) in two short-term animal models: intraperitoneal injection in mice, and inhalation in rats. Both cellulose fibres and the positive control fibre, crocidolite asbestos, were administered in doses ranging from 10^4 to 10^8 fibres and caused marked, dose-dependent recruitment of inflammatory cells to the mouse peritoneal cavity, which was highest 1 day following injection. Crocidolite was much more active than cellulose, despite the mass dose of cellulose being 66 times greater for an equivalent number of fibres. Inhalation exposure induced an early inflammatory response in rat lungs. In vitro production of the proinflammatory cytokine tumour necrosis factor alpha (TNF-α) by lavaged alveolar macrophages was markedly depressed by the end of the exposure period in cellulose-exposed animals. The cellulose material studied was less inflammogenic than crocidolite, and the extent of the inflammatory response within the lung appeared to decrease with continued exposure over a 14-day period (Cullen et al., 2000).

Also, Morgan et al. (2004) reported transient inflammation in the lungs of rats and increased 4-hydroxyproline levels after intratracheal exposure of rats (5 mg/kg body weight) to

cellulose fibres. Microscopic evaluation revealed a minimal to mild, non-progressing granulomatous pneumonitis.

In a study by Adamis et al. (1997), cell count, protein, phospholipid, LDH and acid phosphatase were determined in BAL fluid 1, 3 and 7 days after intratracheal instillation of rats. Interstitial oedema as well as the initial signs of inflammation could be detected in the lung after the first day. Inflammation after 1 week could be noted, partly interstitial and partly intra-alveolar and intrabronchial. In the BAL fluid, protein, LDH, acid phosphatase, phospholipid and cell count were enhanced after days 1 and 3. After 1 month, the developing bronchoalveolitis is fibrous in character. In vitro, cellulose did not damage rat peritoneal macrophages (Adamis et al., 1997).

Cellulose after a single intratracheal dose (15 mg per animal) brought about fibrosing granulomatous alveobronchiolitis and an increase of IgA production in the BAL (Tatrai et al., 1996). Fibrosing alveolitis showed moderate progression as a function of time. With different morphological methods, injury of type I pneumocytes and the incomplete repair of type II pneumocytes were detected by the investigators. The damage of the alveolar epithelium initiated and activated a series of processes that led to definite pulmonary alterations: pulmonary fibrosis leading to the disintegration of the alveolo-capillary morphological functional unit (Tatrai et al., 1996).

Milton et al. (1990) studied the ability of cellulose dust to produce emphysema following intratracheal injection in hamsters. The results showed fibrosing granulomas and patchy thickening of alveolar septa in cellulose-treated animals.

The intratracheal injection of cellulose was also used by Tatrai et al. (1992, 1995), who injected cellulose fibres from material designed for TLC into rats at a dose level of 15 mg. Peribroncheolar granulomas developed in the rat lungs, which were becoming fibrosed after 3 months. As part of these studies, human blood leukocytes were treated with cellulose fibres in vitro. This material caused significant dose-related release of reactive oxygen intermediates.

In a study by Hadley et al. (1992), rats were treated by inhalation for 28 days with an aerosol generated from cellulose building insulation, of which approximately 40% was respirable by rats. Target dose levels were 100, 500 and 2000 mg/m^3; animals were sacrified at the end of the inhalation period when dose-related pulmonary changes were found. The authors observed diffuse macrophage infiltration throughout the pulmonary parenchyma with macrophages enlarged and with foamy cytoplasms. Some areas showed alveolitis and epithelial cell hyperplasia, with some areas consolidated with granulation tissue. Within granulomas, there was evidence of collagen deposition.

Carcinogenicity studies

Rinsky (1990) reported a case–control study of 299 malignancies among paperwool workers and found that the only significant excess for neoplasms was for those of the haematopoietic system. Solet et al. (1989) undertook a proportional mortality analysis of 1010 deaths of workers in the pulp and paper industry. These authors reported a general excess of cancer, largely lung cancer, but did not have information on smoking habits. Jarvholm et al. (1988), Ericsson et al. (1988) and Thoren et al. (1989) reported studies on the same soft paper mill production unit. The odds ratios for mortality from chronic obstructive pulmonary disease and from asthma among the exposed workers were significantly increased. There was no

excess in malignancies. A morbidity study demonstrated a dose-related irritation of the upper respiratory tract. A decrease in lung vital capacity was associated with long-term exposure. Increased elastic recoil pressure and a decreased residual volume were reported among the exposed workers. These findings were considered by the authors to be non-specific reactions to the heavy exposure to paper dust in the mill.

Summary on the determinants of carcinogenic potency

It can be stated that there is evidence that cellulose dust, once it reaches the lung tissue, has significant biological activity, which can include the stimulation of pulmonary fibrosis (Davis, 1996).

It seems that cellulose fibres are more persistent in the lung than *p*-aramid RFP.

References

[References being compiled]

2.2.4.4 Physicochemical properties and biopersistence

Small-diameter cellulose fibres are respirable. There is no information available on the chemical properties of these fibres.

Cellulose fibres are persistent in the lung.

2.2.5 Graphite whiskers (Table 2.5)

2.2.5.1 Epidemiological studies

No data are available.

2.2.5.2 Animal studies

Carcinogenicity by inhalation

No valid study is available (in the only existing study, no tumours were observed, but the fibres were not respirable).

Carcinogenicity by intraperitoneal injection

No data are available.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

In two 16-week studies with rats, no fibrosis was detected at an exposure concentration of 20 mg/m^3 (fibre number not stated; fibre diameters were 3.5 and 7 μm).

Proliferation

No data are available.

2.2.5.3 Mechanistic data

No data are available.

2.2.5.4 Physicochemical properties and biopersistence

Graphite fibres are crystalline and hydrophobic. They have a long half-time in the lungs, with a clearance of >1 year.

2.2.6 Magnesium sulfate whiskers

2.2.6.1 Epidemiological studies

No data are available.

2.2.6.2 Animal studies

Carcinogenicity by inhalation

No tumours were induced in a long-term study (1 year of exposure, observation period total 2 years; no fibre numbers given, but the concentration was 1.4 mg/m^3) in rats.

Carcinogenicity by intraperitoneal injection

No data are available.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

No fibrosis or metaplasia was observed in the inhalation study described above.

Proliferation

No data are available.

2.2.6.3 Mechanistic data

Clearance and changes in situ of the deposited fibres/biopersistence

According to Fujino et al. (1995), magnesium sulfate whiskers are more soluble in vitro than are glass fibres, ceramic fibres and potassium octatitanate whiskers.

Modes of action of fibres in pulmonary/pleural carcinogenesis

In a study by Hori et al. (1994), Wistar rats were exposed to two types of magnesium sulfate whiskers by inhalation for 6 h/day, 5 days/week, for 4 weeks (subchronic study) or for 1 year

(chronic study) to clarify the biological effects of the whiskers. There were few whiskers detected in the rat lungs even at 1 day after the exposure, suggesting that they are dissolved and eliminated rapidly from the lungs. To measure the clearance rate of the whiskers from the lungs, an intratracheal instillation was performed in golden hamsters. The half-life of the whiskers in the lung was determined as 17.6 min by temporally measuring the magnesium concentration up to 80 min after the instillation. A histopathological examination indicated a frequent occurrence of adenoma and carcinoma in the year after chronic exposure, but it was not significantly different between exposed and control rats.

Genotoxicity

No data are available.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

No data are available.

Chronic inflammation, release of cytokines, growth factors, reactive species

Culture of alveolar macrophages with fibres at concentrations of 12.5–100 µg/ml did not induce a significant increase in TNF production and did not significantly affect LDH release (Fujino et al., 1995).

Short-term animal tests

No data are available.

Carcinogenicity studies

No data are available.

Summary on the determinants of carcinogenic potency

There are few data available on the toxicity of magnesium sulfate whiskers. It seems that this fibre is easily soluble and not very persistent in the lung, and it does not induce increased biological responses compared with the negative control.

References

[References being compiled]

2.2.6.4 Physicochemical properties and biopersistence

Magnesium sulfate whiskers have a length of >5 µm and a diameter of <0.5 µm. They are highly soluble, with a half-life of <1 h. They are partially soluble in Gamble's solution.

2.2.7 Polyethylene fibres (Table 2.7)

2.2.7.1 Epidemiological studies

No data are available.

2.2.7.2 Animal studies

Carcinogenicity by inhalation

No data are available.

Carcinogenicity by intraperitoneal injection

No data are available.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

No data are available.

Proliferation

No data are available.

2.2.7.3 Mechanistic data

No data are available on polyethylene fibres. Data are available only on polyethylene dust.

Clearance and changes in situ of the deposited fibres/biopersistence

No data are available.

Modes of action of fibres in pulmonary/pleural carcinogenesis

No data are available.

Genotoxicity

No data are available.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

No data are available.

Chronic inflammation, release of cytokines, growth factors, reactive species

In an in vitro cytotoxicity study by Styles & Wilson (1973), non-fibrous polyethylene dust was found to be less toxic to macrophages than was chrysotile.

Short-term animal tests

No data are available.

Carcinogenicity studies

No data are available.

Summary on the determinants of carcinogenic potency

Almost no data are available on the toxicity of polyethylene fibres.

References

[References being compiled]

2.2.7.4 Physicochemical properties and biopersistence

Polyethylene fibres are produced at lengths above 10–15 μm.

2.2.8 Polypropylene fibres (Table 2.8)

2.2.8.1 Epidemiological studies

Occupational cohort studies of workers exposed to polypropylene raised concerns regarding colorectal cancer in workers. This issue has been addressed in a few epidemiological studies of occupational cohorts engaged in the extrusion of polypropylene fibres. Lewis et al. (1994), in an update of an earlier investigation that reported a 6-fold excess in colorectal cancer in a polypropylene manufacturing plant in Texas, USA, concluded that there was no suggestion of an occupationally related risk. The cohort was small, however, consisting of only 412 male workers. Another small study (Cowles et al., 1994) of a cohort of 257 male employees of a plastics and resins research facility in New Jersey, USA, reported no increased risk of colorectal cancer, although it noted a significant increase in pancreatic cancer and a non-significant increase in lung cancer. It is not clear that the workers were exposed to fibres, and no information on smoking was available.

Goldberg & Theriault (1994) performed a much larger retrospective cohort study in a synthetic textiles factory in Quebec, Canada. The cohort consisted of 7487 men and 2724 women who had been employed for at least 1 year. For men, the risk of colon cancer increased with duration of employment in the polypropylene and cellulose triacetate extrusion unit.

In a cross-sectional study of respiratory effects among 50 workers exposed to polypropylene flock and 45 controls, early signs of interstitial lung disease were noted in high-resolution computed tomography (Atis et al., 2005).

2.2.8.2 Animal studies

Carcinogenicity by inhalation

No data are available.

Carcinogenicity by intraperitoneal injection

No data are available.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

The pulmonary toxicity of polypropylene was tested by Hesterberg et al. (1992) in male Fischer 344 rats after 90 days of inhalation exposure. A recovery group was examined 30 days after the end of the exposure. The polypropylene fibres were size-selected to have a geometric mean diameter of 1.2 μm (geometric standard deviation [GSD] 0.3) (46% <1 μmn) and a geometric mean length of 30.3 μm. Three groups of animals were exposed in nose-only inhalation chambers, 6 h/day, 5 days/week, for 90 days to 15, 30 or 60 mg/m^3 of polypropylene or filtered air (negative control). At 90 days, the lung burden was 26×10^6 fibres/lung. No fibrosis was observed in any of the polypropylene-treated groups (Wagner scale 3); in the positive control group, treated with RCF, the Wagner scale was 4.0.

Warheit et al. (1999) described a study in which rats were exposed to polypropylene fibres (average diameter: 1.6 μm; average length: 20 μm). The rats developed dose- and time-dependent lung changes characterized by increased cellularity and early bronchiolitis, but no fibrosis.

Proliferation

No data are available.

2.2.8.3 Mechanistic data

Fibre diameter depends on the manufacturing process and can vary from >153 μm in monofilament yarn to an average of 1–5 μm in microfibres (ICF, 1986).

Clearance and changes in situ of the deposited fibres/biopersistence

Hesterberg et al. (1992) showed that the number of partially degraded (segmented) polypropylene fibres within the lung increased with the exposure concentration and period of exposure, as well as with the period of recovery after termination of exposure at 90 days. The authors concluded that polypropylene fibres are not fibrogenic to rats after 90 days of inhalation exposure. Polypropylene fibres produced dose-dependent minimal or mild increases in cellularity in lungs, which appear to be reversible after 90 days, especially at the lower dose administered (see above).

Modes of action of fibres in pulmonary/pleural carcinogenesis

No data are available.

Genotoxicity

No data are available.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

No data are available.

Chronic inflammation, release of cytokines, growth factors, reactive species

The pulmonary toxicity of polypropylene was tested by Hesterberg et al. (1992) in male Fischer 344 rats after 90 days of inhalation exposure. The polypropylene fibres were size-selected to have a geometric mean diameter of 1.6 μm (46% <1 μm) and a geometric mean length of 30.3 μm. Three groups of animals were exposed in nose-only inhalation chambers, 6 h/day, 5 days/week, for 90 days to 15, 30 or 60 mg/m^3 of polypropylene or filtered air (negative control). No fibrosis was observed in any of the groups.

In an in vitro cytotoxicity study by Styles & Wilson (1973), non-fibrous polypropylene dust was found to be less toxic to macrophages than was chrysotile.

Short-term animal tests

Warheit et al. (1999) described a study in which rats were exposed to polypropylene fibres (average diameter: 1.6 μm; average length: 20 μm). The rats developed dose- and time-dependent lung changes characterized by increased cellularity and early bronchiolitis, but no fibrosis. The cellular changes were considered to be reversible.

Carcinogenicity studies

No data are available.

Summary on the determinants of carcinogenic potency

It seems that polypropylene fibres are able to induce lung changes characterized by increased cellularity and early bronchiolitis. These changes seem to be reversible after some months. No fibrosis was observed in animal experiments.

References

[References being compiled]

2.2.8.4 Physicochemical properties and biopersistence

In a subchronic toxicity study, the diameter of polypropylene fibres was 1–5 μm, and the length was approximately 30 μm.

Polypropylene fibres exhibit high biopersistence (200 days) by the intratracheal route (mean diameter 0.5 μm, mean length 12 μm).

2.2.9 Polyvinyl alcohol fibres (Table 2.9)

2.2.9.1 Epidemiological studies

No data are available.

2.2.9.2 Animal studies

No data are available.

2.2.9.3 Mechanistic data

Clearance and changes in situ of the deposited fibres/biopersistence

The diameter of PVA fibres, as manufactured, is above the respirable limit, and most of them are not inhalable. They have a lower density as mineral fibres. The fibres are mostly in the range of 10–16 μm in diameter. There is evidence that they do not fibrillate (Harrison et al., 1999).

Modes of action of fibres in pulmonary/pleural carcinogenesis

Morinaga et al. (1999) did not observe an increased lung cancer risk in workers exposed to PVA fibres.

Genotoxicity

PVA compounds were not genotoxic in a range of in vivo and in vitro studies (Table 2.10).

Table 2.10 Genotoxicity of PVA compounds in vitro and in vivo

End-point	Test system	Test material	Concentration	Results	Reference
In vitro					
Reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537	PVA	5000 mg/plate	Negative	Huntingdon Life Sciences (2000a)
Reverse mutation[b]	*S. typhimurium* TA1537	PVA	7500 mg/plate	Negative	Huntingdon Life Sciences (2000a)[e]

End-point	Test system	Test material	Concentration	Results	Reference
Reverse mutation[a]	*Escherichia coli* WPA *uvrA*/ pKM101	PVA	5000 mg/plate	Negative	Huntingdon Life Sciences (2000a)[c]
Cell mutation[a]	Mouse lymphoma L5178Ycells, $Tk^{+/-}$ locus	PVA	5000 mg/ml	Negative	Huntingdon Life Sciences (2000b)[c]
In vivo					
Micronucleus formation	Mouse bone marrow	PVA	2000 mg/kg	Negative	Huntingdon Life Sciences (2000c)[c]

[a] In the absence and presence of metabolic activation from S9 liver microsomal preparations.
[b] In the presence of metabolic activation from S9 liver microsomal preparations.
[c] Study conducted in accordance with United States Department of Agriculture Good Laboratory Practice (GLP) regulations (Part 58 of 21 Code of Federal Regulations), European Economic Community GLP regulation 99/11/EEC and Organisation for Economic Co-operation and Development GLP principles.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

No data are available.

Chronic inflammation, release of cytokines, growth factors, reactive species

No data are available.

Short-term animal tests

No data are available.

Carcinogenicity studies

No data are available.

Summary on the determinants of carcinogenic potency

PVA fibres, as manufactured, are above the respirable limit, and most of them are not inhalable. The only study on lung cancer risk in workers exposed to PVA fibres did not show positive results. PVA itself is not genotoxic.

References

[References being compiled]

2.2.9.4 Physicochemical properties and biopersistence

The diameter of PVA fibres is mostly in the range of 10–16 μm. They have a lower density as mineral fibres. The respirable limit is about 7 μm. The fibres do not fibrillate.

There are no data on biopersistence.

2.2.10 Polyvinyl chloride fibres (Table 2.11)

2.2.10.1 Epidemiological studies

There are no epidemiological studies of polyvinyl chloride (PVC) fibres. The studies by Mastrangelo et al. (2005) and earlier studies by Wu et al. (1989) and Waxweiler et al. (1981) reflect exposure not to fibres but rather to granular particles.

2.2.10.2 Animal studies

No data are available on fibrous PVC.

2.2.10.3 Mechanistic data

No data are available on PVC fibres. Data are available only on PVC dust and vinyl chloride monomer (VCM).

Clearance and changes in situ of the deposited fibres/biopersistence

No data are available.

Modes of action of fibres in pulmonary/pleural carcinogenesis

No data are available.

Genotoxicity

Wong et al. (2003) investigated genetic polymorphisms and sister chromatid exchanges in VCM-exposed PVC workers and found that workers with susceptible metabolic and DNA repair genotypes experience an increased risk of DNA damage elicited by VCM exposure.

Lei et al. (2004) investigated DNA single strand breaks in PVC monomer–exposed workers and correlated it to urinary thiodiglycolic acid levels. The results of this study showed that levels of DNA single strand breaks were increased with urinary thiodiglycolic acid levels. The dose–response relationship of urinary thiodiglycolic acid level and DNA single strand breaks was particularly significant among the workers with a urinary thiodiglycolic acid level of 4 mg/g creatinine, which is equivalent to a 5 ppm airborne VCM level. The authors concluded that airborne VCM exposure greater than 5 ppm could induce DNA damage.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

Six samples from the normal industrial suspension process (PVC-S) and eight samples from the emulsion process (PVC-E) were studied for cytotoxicity in vitro using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay, after 20 h of incubation in A549 cells and in primary cultures of alveolar macrophages (AM) and type II pneumocytes (TII) obtained from rats (r-AM, r-TII) or from human surgical specimens (h-AM, h-TII). Haemolysis was assessed after 2 h of incubation with human erythrocytes (h-RBC). A positive control (crystalline silica, Min-U-Sil) and relevant additives of these PVC particles were tested concurrently. No toxicity of PVC-S particles could be established up to 5 mg/ml in the haemolysis test and 2.5 mg/ml in the MTT assay. In contrast, four out of eight PVC-E particles induced significant toxicity, with median effective concentration (EC_{50}) values ranging from 0.7 to 3.7 mg/ml in the haemolysis test and from 0.2 to 1.2 mg/ml in primary cells. The most toxic particles were those that contained the additives with the highest in vitro cytotoxicity. There was a good correlation between EC_{50} values obtained in relevant bioassays. In conclusion, PVC-E particles produced a moderate in vitro toxicity for primary rat and human pulmonary cells, probably because of the residual presence of additives (Xu et al., 2002).

To elucidate the role of residual additives, two of the emulsion PVC particles were chosen for a follow-up study (Xu et al., 2003). Here, the authors compared the cytotoxicity and the release of cytokines by PVC particles with their "additive-free"counterparts and with the pure additives sodium lauryl sulfate and sodium alkylbenzenesulfonate. The authors found that unwashed PVC particles exhibited the potential to damage pulmonary cells and to induce the release of inflammatory mediators in vitro. These particles might also have hazardous potential for the lung under excessive occupational exposure. Xu et al. (2003) proposed that the hazardous potential of these PVC particles is related to their residual additives.

Chronic inflammation, release of cytokines, growth factors, reactive species

Enzymatic and pathomorphological alterations in rat lungs were studied by Agarwal et al. (1978) at different time intervals up to 180 days after a single intratracheal administration of 25 mg of PVC dust. The activities of two energy-linked enzymes, succinate dehydrogenase (SDH) and adenosine triphosphatase (ATPase), and three lysosomal enzymes, acid phosphatase, β-glucuronidase and ribonuclease, were significantly increased in the early period and then started to decline. The activities of SDH and ATPase reached control values at 150 days, whereas those of the lysosomal enzymes remained significantly higher up to this period. Histopathologically, the pulmonary response was in the form of acute inflammatory changes during the early stages of dust burden, followed by the development of granulomatous lesions containing small amounts of stromal elements.

Short-term animal tests

Rats, guinea-pigs and monkeys were exposed in a study by Groth et al. (1981) by inhalation (6 h/day, 5 days/week) for up to 22 months to a 13 mg/m^3 concentration of PVC dust.

Autopsies on rats and guinea-pigs were performed after 12 months of exposure and on monkeys after 22 months of exposure. Lung function tests were performed on monkeys after 9, 14 and 22 months of exposure. Aggregates of alveolar macrophages containing PVC particles were found in the lungs of all animals. These aggregates were more numerous in the monkey lungs. No fibrosis or significant cellular infiltrates were present in or near these cellular aggregates. No significant effects on pulmonary function could be demonstrated in the monkeys exposed to PVC. Under the conditions of this experiment, inhaled PVC produced a benign pneumoconiosis.

Also, Wagner & Johnson (1981) did not observe carcinogenic effects in rats after inhalation or intrapleural injection of 20 mg PVC dust.

PVC dust, following a single intratracheal instillation (25 mg/rat), was substantially cleared through the lymphatic circulation and progressively accumulated in the tracheobronchial lymph nodes in studies by Agarwal et al. (1991). The tissue response in tracheobronchial lymph nodes during 60–270 days post-instillation of PVC dust was characterized by progressive increase in total organ fresh weight, dry weight, DNA, ribonucleic acid (RNA) and protein contents, concurrent with the proliferation of macrophages and hyperplasia of reticular cells. Active phagocytosis and enhanced hydrolytic activity in tracheobronchial lymph nodes was evident around 270 days post-instillation by the appearance of PVC-laden macrophages near and within the dust foci and increased activity of acid phosphatase, deoxyribonuclease (DNase), ribonuclease (RNase) and β-glucuronidase. PVC dust caused degeneration of macrophages, and consequent release of hydrolytic enzymes resulted in limited cytotoxicity without inducing reticulination and fibrosis in the tracheobronchial lymph nodes. The histology and clinical biochemistry of liver, kidney, spleen and serum were not altered, and there were no detectable PVC particles in these tissues at up to 365 days. The authors therefore concluded that lymphatic clearance of intratracheally instilled PVC dust results in its accumulation and mild foreign body reaction in tracheobronchial lymph nodes, which is non-fibrogenic at up to 365 days post-instillation (Agarwal et al., 1991).

In vitro studies of Xu et al. (2002, 2003) indicated that emulsion PVC particles (PVC-E3), with a mean diameter of 2 μm exhibited a moderate toxicity in different pulmonary cell cultures. The in vitro cytotoxicity and proinflammatory potential of PVC-E3 particles were reduced when the additives had been "washed off" (PVC-W3), indicating that PVC particle–associated toxicity is probably related to the residual additives. The in vivo study of Xu et al. (2004a) with male Wistar rats did not confirm the conclusion from the in vitro toxicity tests. The pulmonary toxicity of both PVC-E3 and PVC-W3 particles appeared limited. In a follow-up study by Xu et al. (2004b), rats were repeatedly intratracheally instilled with PVC particles, but the results were similar to those with single instillations. The examined PVC particles had the potential of inducing a limited and transient acute inflammatory reaction in the lung and possibly a more persistent alteration of pulmonary T lymphocyte subsets towards a high CD4/CD8 ratio.

Carcinogenicity studies

In 1974, vinyl chloride (VC) was first reported in the scientific literature to induce angiosarcoma of the liver both in humans and in animals. Additional research has demonstrated the carcinogenicity of VC to other organs and at lower concentrations. The target organs for VC now clearly include the liver, brain, lung and probably the lymphohaematopoietic system. The evidence for a carcinogenic risk has been extended to

jobs associated with PVC exposure. Cases of liver angiosarcoma have been reported among individuals employed in PVC fabrication facilities, and an epidemiological study has demonstrated a significant association between exposure to PVC dust and the risk of lung cancer mortality. Cases of angiosarcoma of the liver also have been reported among individuals living in close proximity to VC/PVC plants. An association between PVC dust and pneumoconiosis has also been demonstrated. On the basis of findings, prudent control of PVC dust in the industrial setting is indicated (Wagoner, 1983).

Overall, the results of the analysis of 12 studies of VC production and polymerization workers demonstrate an elevated risk of liver malignancies and the possibility of a 2-fold increased risk of brain and central nervous system tumours and perhaps, also, of malignancies of the lymphatic and haematopoietic system (Nicholson et al., 1984). However, the role of other agents cannot be excluded in the etiology of non-hepatic malignancies. Bronchogenic carcinoma does not appear to be increased from exposures to VCM, although a relationship to PVC dust was suggested in one study. These conclusions must be considered in light of limited data on workers followed more than 25 years from onset of exposure. Considering the numbers of observed and expected deaths in all studies, it would appear that the excess of malignancies at non-hepatic sites is less than the excess of liver tumours. To the extent that VC exposure is associated with other cancers, a similar risk reduction would be expected. Raynaud's phenomenon, acro-osteolysis, scleroderma-like skin lesions, hepatomegaly and splenomegaly with non-cirrhotic hepatic fibrosis and severe portal hypertension have been associated with past heavy exposures to VC. Evidence exists that the liver disease and portal hypertension may progress following cessation of exposure. However, all of the above syndromes were found largely in heavily exposed individuals. Their occurrence would be much less likely in workers exposed only to concentrations currently allowed. Pulmonary deficits, X-ray abnormalities and, perhaps, lung cancer have been associated with VC/PVC exposure (Nicholson et al., 1984).

Increased respiratory cancer was reported by Waxweiler et al. (1981) among a cohort of 4806 males employed at a synthetic chemicals plant. Upon review of pathologic material, the excess was found to be limited to adenocarcinoma and large cell undifferentiated lung cancer. Many of the workers had been exposed to VC, as well as to chlorinated solvents, PVC dust, acrylates and acrylonitrile. PVC dust appeared to be the most likely etiologic agent. Time trends of PVC dust exposure indicated a potential latent period of 5–16 years before death (Waxweiler et al., 1981).

Hsiao et al. (2004) concluded from their studies that PVC workers who had high exposure to VCM have an increased risk of developing liver fibrosis.

Several authors reported that occupational exposure to PVC dust is associated with lung disorders called "PVC pneumoconiosis" (Szende et al., 1970; Arnaud et al., 1978; Antti-Poika et al., 1986; Studnicka et al., 1995). Epidemiological studies indicated that occupational PVC dust exposure might affect pulmonary function and lead to a higher prevalence of small opacities on chest X-ray (Soutar & Gauld, 1983; Lloyd et al., 1984; Nielsen et al., 1989; Lee et al., 1991; Ng et al., 1991).

Pott et al. (1989) reported that large amounts of PVC dust led to adhesions of the abdominal organs and fibrosis, but a definite carcinogenic effect was not detected.

Summary on the determinants of carcinogenic potency

It seems likely that workers in the PVC industry who were exposed for a longer time to high PVC levels (>10 mg PVC/m^3) have an increased risk of developing pneumoconiosis and decreased lung function. Effects were observed just after long-term exposure to PVC dust.

According to Nicholson et al. (1984), the risk of VC/PVC-induced malignancies has decreased after 1974 because of improved technologies in the PVC industry.

In animal experiments with PVC dust, most studies did not show significant cellular effects (no fibrosis, no significant effects on pulmonary function, no carcinogenic effects in rats). However, in vitro studies in several cellular systems showed cytotoxic and genotoxic effects. It might be that these effects are reversible and non-mutagenic in vivo.

References

[References being compiled]

2.2.10.4 Physicochemical properties and biopersistence

No data are available.

2.2.11 Potassium octatitanate fibres (Table 2.12)

2.2.11.1 Epidemiological studies

No data are available.

2.2.11.2 Animal studies

Carcinogenicity by inhalation

In a 2-year inhalation (up to 200 WHO fibres/ml) study in rats, no tumours were observed. The observation period thus was short and the exposure concentration too low for reliable detection of tumour induction.

In another 2-year study (1-year exposure, 1-year additional follow-up) in rats, no tumours were observed.

Carcinogenicity by intraperitoneal injection

There exist four intraperitoneal injection studies: one in hamsters and three in rats. In all studies, a dose-dependent increased incidence of mesothelioma was observed (Stanton, 1978; Lee et al., 1981; Pott et al., 1989; Adachi et al., 2001). Up to 80% animals had mesothelioma after exposure to 10 mg.

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

In the first 2-year inhalation study in rats described above, no fibrosis was observed, whereas in the other, mild fibrosis was reported.

Proliferation

No data are available.

2.2.11.3 Mechanistic data

There is a small database; DNA strand breaks and chromosomal aberrations have been observed. There is evidence of strong genotoxicity, comparable to that of chrysotile.

2.2.11.4 Physicochemical properties and biopersistence

The fibres have low solubility and small diameters. Iron and cobalt are present as impurities.

The fibres have a long half-life.

2.2.12 Synthetic vitreous fibres (Table 2.13)

2.2.12.1 Epidemiological studies

General introduction: glass wool, continuous glass filament, and rock (stone) and slag wool during manufacture

Two large cohort studies and case–control studies nested within these cohorts from the USA and Europe provide most of the epidemiological evidence concerning potential risk for respiratory and other cancers associated with occupational exposure to glass wool, continuous glass filament and rock (stone)/slag wool during manufacture. The cohort study from the USA included 16 plants, extended the follow-up to 1992 and expanded a previous cohort to include women and non-white workers. This study included information on smoking habits and a new assessment of historical workplace exposure to respirable fibres and several sources of co-exposure, including asbestos, formaldehyde and silica. The European cohort extended the follow-up to 1990 in 13 plants.

Glass wool

The findings of the cohort study from the USA provided no evidence of excess mortality from all causes combined or from all cancers combined, using local rates. A statistically significant 6% excess in respiratory cancer (primarily trachea, bronchus and lung) mortality was observed. When analysis was restricted to long-term workers, the excess was reduced and was no longer statistically significant. Adjustment for smoking based on a random sample of workers suggests that smoking may account for the excesses in respiratory cancer observed in the male glass fibre cohort (glass wool and continuous glass filament combined). The SMRs for respiratory cancer were related neither to duration of employment among the total cohort or among long-term workers nor to duration of exposure, cumulative exposure or average intensity of exposure to respirable glass fibre (glass wool and continuous glass

filament combined). Analysis by product group showed a statistically significant excess of respiratory cancer for all workers from plants grouped as "mostly glass wool", but this excess risk for the "mostly glass wool" product group was reduced and no longer statistically significant when the cohort was limited to long-term workers (≥5 years of employment). There was no evidence of an excess of mesothelioma or non-respiratory cancers. The case–control study of respiratory cancer nested within the United States cohort enabled control of plant co-exposure and a more detailed control for confounding by smoking. Duration of exposure, cumulative exposure, average intensity of exposure and the time since first exposure to respirable glass fibre were not associated with an increased risk of respiratory cancer. These results were not altered by using different characterizations of categorized respirable fibre exposure or alternative models for continuous exposure data.

The European cohort study of glass wool workers demonstrated an increased mortality from lung cancer (trachea, bronchus and lung) but no trend with time since first hire or duration of employment. One death from mesothelioma was observed in this cohort. This study did not estimate fibre exposure, but used surrogate measures such as "technological phase at first employment". No information was available either on co-exposure or on smoking habits.

Continuous glass filament

Two of the plants of the cohort study from the USA manufactured only continuous glass filament. For all workers and for long-term workers from these two plants, no evidence of excess mortality from respiratory cancer was found when compared with local rates. Adjustment for smoking had little effect on the SMR for respiratory cancer. A nested case–control study that included adjustments for smoking and co-exposure also provided no consistent evidence of excess mortality from respiratory cancer. The exposure–response analyses that combined exposures to continuous glass filament and to glass wool are reported in the section on glass wool.

The European cohort study reported few data to evaluate cancer risks among workers exposed to continuous glass filament. This study provided no convincing evidence of an elevated risk for lung cancer.

Results were also available from two smaller cohort studies in the USA and Canada. The cohort study on one continuous glass filament plant in the USA, which included a nested case–control study, with information on smoking and co-exposure, provided no consistent evidence of an excess risk for lung cancer. The Canadian cohort study of one continuous glass filament plant did not include an assessment of smoking or co-exposure. This study also provided no consistent evidence of an excess risk for lung cancer.

Rock (stone) and slag wool

The present evaluation relies mainly on cohort and nested case–control studies, in which exposure to rock (stone) wool and exposure to slag wool were not considered separately.

The extended follow-up of the rock (stone)/slag wool cohort from the USA indicated an overall elevated risk of respiratory cancer when either national or local comparison rates were used. However, no association was found with duration of exposure or with time since first exposure. SMRs were no longer elevated when indirect adjustment for smoking was made. The nested case–control study showed no association between respiratory cancer and

estimated cumulative exposure to respirable fibres, with or without adjustment for possible confounding by smoking and other sources of occupational exposure. Another nested case–control study partially overlapping with the study in the USA showed no increased risk for respiratory cancer in association with exposure to slag wool.

The extended follow-up of the European cohort study indicated an overall elevated risk for lung cancer when national comparison rates were used. This study showed an increasing risk with years since first exposure. The highest SMR was found among workers with the longest time since first employment and among those first employed in the "early technological phase" (i.e. before the introduction of oil and binders and use of the batch processing method). However, in a case–control study that included detailed information on exposure to fibres, individual smoking habits and potential occupational confounders, no increased risk of lung cancer with increasing fibre exposure was reported.

The results from these studies provide no evidence of an increased risk of pleural mesotheliomas or any other tumours.

Refractory ceramic fibres

Preliminary results from a epidemiological mortality study of RCF workers in the USA were available. However, the limited epidemiological data do not permit an adequate evaluation of the cancer risk associated with exposure to RCF.

A cohort study of workers at two plants in the USA that produced RCF, which included 927 male workers employed for 1 year or more between 1952 and 1997, was described by LeMasters et al. (2001) and in a paper addressing risk analysis (Walker et al., 2002). The estimated exposure ranged from 10 fibres/cm^3 (8-h time-weighted average) in the 1950s to <1 fibre/cm^3 in the 1990s. No significant increase in cancer mortality was reported. (The working group noted that neither the observed nor the expected numbers of cancers other than lung cancer were given.) Six deaths from lung cancer were observed versus 9.35 expected (SMR = 0.64; 95% CI = 0.24–1.27). No cases of mesothelioma were observed. (The working group noted that the details of cohort definition and period of follow-up were not clear, and there was no analysis of risk in relation to time since first exposure or exposure surrogates. The small number of study subjects, especially those with adequate latency, limits the informativeness of the study.)

An update of the study of RCF workers in the USA has been published (LeMasters et al., 2003) since the completion of the IARC MMVF monograph in 2002. This study included the same group of RCF workers who were first employed between 1952 and 1 June 1987 that was included in the initial study (LeMasters et al., 2001) as well as workers who were first employed from 1 June 1987 until 1 January 2000. The study was limited to workers with at least 1 year of employment, resulting in a final study size of 942 workers. The study extended the follow-up for vital status ascertainment to 31 December 2000, resulting in the identification of 87 deaths from all causes and 29 deaths from lung cancer. A deficit in mortality from respiratory cancer was observed based on a life table analysis using either the population of the USA (SMR = 82.5, 95% CI = 37.7–156.7) or the New York State population (SMR = 87.5, 95% CI = 37.7–172.5) as the reference group. An analysis using the Cox proportional hazards model failed to demonstrate a trend between cumulative exposure to RCF fibres and lung cancer risk. No cases of either pleural cancer or mesothelioma were identified in this study based on a review of the death certificates. Five of the deaths were

known to have pleural plaques, and five had interstitial changes based on information collected in a more extensive morbidity study (Lockey et al., 2002).

A critical issue in interpreting the findings from the LeMasters et al. (200?) study is whether or not the study had adequate power to detect an excess risk of mesothelioma or lung cancer. The authors of the study suggest that their study was limited by its relatively small size, relative youth (mean age = 51 years) and possible non-comparability with the general population. They suggest that the cohort has achieved "a reasonable period of time since hire" with a mean latency (time since first exposure) of over 21 years. However, it should be noted that the mean latency implies that a substantial fraction of the cohort would have less than 21 years of follow-up and furthermore that, based on our experience with asbestos, 21 years may not be an adequate period of time, particularly for mesothelioma. Based on the study size, the authors estimated that this cohort had only a 40% chance (i.e. statistical power) to detect a 2-fold increase in lung cancer risk.

In the paper by Walker et al. (2002), an analysis of the same cohort was presented in which an attempt was made to test whether or not the findings from the study were statistically inconsistent with what would be expected for asbestos. Expected numbers of lung cancer were derived by applying models that were developed by Hodgson & Darnton (2000) for lung cancer and mesothelioma. Background rates of lung cancer in the population of the USA were used with the models to estimate the expected deaths for lung cancer. Their findings indicated that the observed lack of mesothelioma deaths in the LeMasters et al. (200?) study was not statistically inconsistent with what would be expected based on the potency of asbestos. For lung cancer, they reported that the results from the LeMasters et al. (200?) study were statistically inconsistent with what would be expected based on the potency for crocidolite or amosite, but that an effect consistent with the potency for chrysotile could not be ruled out.

The working group noted two issues that limit their confidence in the authors' conclusions with regard to lung cancer. First, the expected number of deaths for the analysis was based on the population of the USA. As LeMasters et al. (200?) suggested, this may be an inappropriate referent group for this study population. The SMR for lung cancer was less than 100 for lung cancer (SMR = 82.5, 95% CI = 37.7–156.7) when comparisons were made with the population of the USA. This may suggest that the RCF workers in this study were a select group who were healthier than the general population (i.e. healthy worker effect). The other issue that is not addressed by the Walker et al. (2002) analysis is the relatively incomplete follow-up of the RCF cohort. The models for lung cancer that were used by Walker et al. (2002) come from studies that are likely to have had much longer follow-up than the LeMasters et al. (200?) cohort of RCF workers. If this is the case, then an adjustment for the shorter follow-up (if this could be accomplished) of the LeMasters et al. (200?) cohort would result in a smaller number of expected lung cancer deaths, and thus the differences between the predicted and observed numbers would be diminished.

Radiographic evidence indicating pleural plaques has been reported for RCF workers. Although the prognostic significance of pleural plaques is unclear, such plaques are also a common finding among asbestos-exposed workers.

Man-made vitreous fibres (not otherwise specified)

A number of studies did not separate exposure to glass wool from exposure to rock (stone) and slag wool or other fibre types or had limited ability to distinguish between these different fibre types. Since much more information was available from epidemiological studies in the fibre production industries, no separate evaluation is made for the studies of mixed exposure. The results of these studies were, however, taken into consideration for the evaluation of the distinct fibre types.

A cohort study of Swedish wooden house industry workers exposed to MMVF demonstrated a decreased risk for lung cancer and no positive trend in SMRs for lung cancer with duration of employment. An increased risk for stomach cancer was found, but the risk did not increase with duration of employment.

Two population-based case–control studies in Germany were combined in a pooled analysis that suggested an association between lung cancer and occupational exposure to MMVF. Odds ratios were adjusted for smoking and exposure to asbestos, but exposure to MMVF and asbestos may not have been separated well enough to rule out residual confounding as an explanation of the results. A low response rate in one of the reference groups adds to the uncertainty of the validity of this study.

A population-based case–control study from Canada found no association between lung cancer and occupational exposure to glass wool or rock (stone) and slag wool.

A German case–control study suggested an association between mesothelioma and exposure to MMVF adjusted for asbestos exposure. However, several limitations constrain the interpretation of the reported results, particularly the potential for misclassification of exposure to asbestos and MMVF and the small number of cases and controls classified as ever having been exposed to MMVF without exposure to asbestos.

An increased risk for laryngeal and hypopharyngeal cancer in association with exposure to MMVF was reported in a case–control study from France, but this was an isolated finding not observed in other studies.

2.2.12.2 Animal studies

From the toxicological/biological point of view, it makes little sense to distinguish between the fibre types listed above, as they are all vitreous and similar in chemical composition. Fibre dimensions and the relative amounts of the individual chemical components may vary to a large degree, and this may lead to dramatic differences in the pattern of deposition and biological and toxicological response. This can most easily be shown by differences in the biopersistence of the different fibres.

Carcinogenicity by inhalation

Some of the fibres tested showed a clear-cut positive response (E-glass, RCF1)—that is, they induced lung tumours in rats. Both E-glass and RCF1 contained granular particles, and it cannot be excluded that these granular particles contributed to the induction of the lung tumours. It was also possible to get an indication of differing tumorigenic potency (E-glass more potent than 475). Other fibres (e.g. MMVF10, MMVF11, MMVF21, MMVF22) did not

induce tumours, but in many of the studies the exposure in regard to fibre concentration (but not necessarily total mass concentration) was low.

Carcinogenicity by intraperitoneal injection

Compared with rat inhalation studies, carcinogenicity testing of fibres by intraperitoneal injection represents a sensitive assay. With this system, it is possible to detect the carcinogenic effect of fibres whose potency is more than 3 orders of magnitude lower than the potency of crocidolite, such as B-01-0.9. There was a close association between the fibre biopersistence in the lung and the carcinogenic potency in the peritoneum. A ranking order of potency (number of fibres with length >5 μm) required to induce tumours at the 25% level was as follows: crocidolite, ceramic, basalt (stone wool), MMVF21 (stone wool), B-20-2.0 (experimental stone wool), M-stone (stone wool); MMVF11 (glass wool), M-slag (slag wool); B-09-2.0 (experimental glass wool); R-stone-E3 (experimental stone wool), B-01-0.9 (experimental glass wool) (Roller & Pott, 1996; TRGS, 1999).

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

Similar biopersistence-related variation in response as seen with the carcinogenic potency was also observed with fibrotic changes in the lung after inhalation exposure.

Proliferation

Proliferation of terminal bronchial epithelium and alveolar parenchymal cells has been observed after 3 months' inhalation exposure to E-glass and MMVF21 and the rock wools RIF 41001 and RIF 42020-6 (Bellmann & Muhle, 2003; Kamstrup, 2004).

2.2.12.3 Mechanistic data

Introduction

Table 2.14 summarizes results collected in the latest IARC Monograph (IARC, 2002), results reported herein and information in Table 2.13.

Table 2.14 Summary of information on clearance, biopersistence and mode of action of synthetic vitreous fibres

Fibre type	Fibrous glass	Mineral wool	Ceramic fibres
Clearance/biopersistence			
Mucociliary mvt			
Transport (half-time, days)[1]	2–80	5–60	6–50
Pleural translocation	+		+
Dissolution in situ			
Fibre fragmentation in situ			
Mode of action			
Phagocytosis	+	+	+
Point mutations	(+)[2] (−)[2, 3]	(+)	(−)
Base, DNA damage	+	+	+

Fibre type	Fibrous glass	Mineral wool	Ceramic fibres
Deletions			
Effects on mitosis	+	+	+
Chromosome damage	+[4]	+	+[4]
Gene transfer	(−)		
Malignant transformation[5]	+		
Growth alteration			
Altered differentiation			
Activation of growth factors (GFs)			
Activation of growth factor receptor (GFR)			
Signalling pathways			
Death: apoptosis, necrosis			
Cell influx in BAL			
Cytokine release			
Release of growth factors			
ROS production[6]	+	+	+[7]
Short-term animal tests			
Carcinogenicity studies	+	+	+

[1] From McConnell (2000).
[2] In parentheses: one experiment found.
[3] In vivo assay in Big Blue rats; 5 days' exposure to short fibres.
[4] Dependence on fibre dimensions.
[5] In vivo assays with cells in culture (morphological transformation, anchorage independence).
[6] In vivo assays with cells in culture and in vitro assays.
[7] Several assays concluded to low effect.

Since the last IARC monograph publication (IARC, 2002), most of the literature on fibrous glass, mineral wool and ceramic fibres has been based on analyses of nose-only inhalation studies conducted at the Research and Consulting Company (RCC). These papers mainly focused on risk assessment for lung cancer. As far as RCC findings are concerned, results lead to the conclusion of an absence of statistically significant carcinogenic potency of different glass wools (glass fibres MMVF10, MMVF12; slag wool MMVF21) and rock wool MMVF21; and of the carcinogenicity of ceramics RCF1, RCF2 and RCF3, but not RCF4 (Rossiter & Chase, 1995). While the exploitation of such a large number of data is of great interest to formulate hypotheses on the mechanisms of fibre deposition and clearance, and of carcinogenesis, several comments can be made:

- Under different experimental conditions, other studies demonstrated carcinogenic potency with other samples of fibrous glass and mineral wool (IARC, 2002).
- Interpretation of RCC data have been taken from results obtained in one animal species (rat).
- Nose-only exposure is not similar to the situation encountered in human exposure.
- Moreover, it must be emphasized that the risk for mesothelioma, a major concern in the field of fibre exposure, was not taken into consideration regarding the low rate of mesothelioma observed by inhalation in rats and the absence of data on pleural burden.

In the recently published papers, it has been proposed that the half-time of fibres longer than 20 μm is a good indicator of lung cancer risk for inhaled fibres (Moolgavkar et al., 2001;

Daniel Maxim et al., 2002; Turim & Brown, 2003). The cut-off of 20 μm of length for fibres presenting the most biologically active potential is rather hypothetical. It is based on the assumption that macrophages do not ingest longer fibres. While it is well demonstrated that the biological effects of "long" fibres are higher than those of "short" fibres, it does not imply that short fibres have no effect. Fibres 20 μm in length appear to be retained in the nasal compartment. When studying the maximal tolerated dose of RCF in rats, Mast et al. (2000) reported that the mean sizes of RCF fibres were 20.3 μm, 8.13 μm and 7.62 μm for fibres deposited in the nose, tracheobronchial and pulmonary compartments, respectively, for an aerosol with mean fibre length of 15.9 ± 2.4 μm. This study indicates that, by inhalation, fibres longer than 20 μm might be more likely to pose a problem to the nasal compartment in rats and that smaller fibres should be considered to study pulmonary effects. Interestingly, Dorger et al. (2000) found that MMVF10 and MMVF21 fibres phagocytized by rat and hamster alveolar macrophages corresponded to these dimensions, as the median length was about 10 μm in both species. In this assay, the median lengths of the fibres to which cells were exposed were 16.3 μm for MMVF10 and 19.4 μm for MMVF21.

Several models have been applied to interpret RCC data using quantitative risk analysis. A synthesis of quantitative risk analyses for RCF fibres is given by Daniel Maxim et al. (2003). Using a two-stage clonal expansion model, Moolgavkar et al. (1999, 2000) found that a model positing these RCF fibres as an initiator had the highest likelihood. Several years ago, Lu et al. (1988) formulated similar conclusions using a two-step transformation assay with cells in culture. With that in mind, studies investigating genotoxicity are of particular interest. Hence, tumour development in animals seems to be the consequence of fibres' genotoxicity and of inflammatory reactions (otherwise considered a secondary mechanism) and/or of a complete carcinogenic potential of the fibres.

Investigation of inflammation is based on the hypothesis that molecules released during the inflammatory process are taken as the main process accounting for the carcinogenicity of particles. Based on this assumption, the persistence of the particles in the lung is considered as the major relevant parameter to evaluate the carcinogenic potential of fibres. While inflammation contributes to the carcinogenic process, biopersistence of an agent is not a prerequisite for its carcinogenic potency, and it is well evident that carcinogens may be non-persistent agents. Biopersistence can be taken as important, as it determines the dose accumulating in the lung tissue, but it should not be taken as the discriminative parameter of carcinogenicity. It is now established that cancer is a multistep process; inflammation may be one of them, but it is not unique. The long delay between the beginning of exposure and the revelation of the disease is consistent with this mechanism.

In that respect, it is well established that interaction with the genetic material (DNA and chromosomes) is one major critical parameter to account for carcinogenicity. Interaction may take place at the time or early after fibre deposition, leaving a susceptible cell that will exhibit fully transformed phenotype and cancer development later, with the occurrence of other damage and cellular changes. Investigations related to genetic damage provoked by MMVFs are poorly developed. The few studies carried out so far encourage the development of these investigations. In a recent paper, Speit (2002) proposed pertinent assays for genotoxicity testing of fibres.

In a previous IARC issue (IARC, 2002), results obtained with glass fibres were reported. A positive response in genotoxicity tests in non-human mammalian cells was found with several samples (induction of DNA damage, formation of micronucleus and of binucleated and

multinucleated cells), depending on fibre dimensions. Few data were available on the genetic effects of rock wool and slag wool, but some mutagenic effect was found in *Salmonella typhimurium*, as well as DNA and chromosome damage in hamster or human cells. Concerning ceramic fibres, genetic alterations have been detected with some samples in different tests using *S. typhimurium*, *Drosophila melanogaster* and hamster and human cells.

Fibrous glass (glass fibres, glass wool)

Transport of fibres

Adhesion of fibres to cells is a parameter playing a role in the movement of fibres in the respiratory system. An adhesion of MMVF10 to tracheal explants was found to be enhanced with high doses of TNF-α, a cytokine produced during the inflammatory process (Xie et al., 2000).

Glass fibre type B-01/09 (Bayer fibre) has shown a clearance half-time of 37 days, following nose-only exposure of rats (Creutzenberg et al., 1997). Clearance half-time was also calculated from measurements with tracer particles of $^{46}Sc_2O_3$; it was not different from that of control clean air–exposed animals.

Pleural translocation

Experiments carried out by Bermudez et al. (2003) demonstrated that MMVF10a fibreglass was deposited in the lung and translocated to the pleura of both Fischer 344 rats and Syrian golden hamsters after 4 weeks' exposure by nose-only inhalation at nominal aerosol mass concentrations of 45 mg/m^3 (610 WHO fibres/m^3). Pleural burden was significantly greater in hamsters than in rats. Importantly, fibres >8 μm in length were detected in both lung and pleura, demonstrating that fibres other than "short" fibres are found in these tissues after up to 12 weeks of recovery following 12 weeks of exposure. In the pleura, this size category was the most frequent in both species. MMVF10a is a slightly thinner and longer fraction of MMVF10.

Phagocytosis

Internalization of a glass wool sample by MeT-5A mesothelial cells in culture was reported by Cavallo et al. (2004).

MMVF10 fibres were reported to be phagocytized by rat and hamster alveolar macrophages (Dorger et al., 2000). The median sizes of the internalized fibres were 10.4 μm and 8.8 μm in rat and hamster macrophages, respectively. A low cytotoxicity in rat macrophages resulted, but no toxicity was found in hamster macrophages.

In animals, alveolar macrophages showed phagocytosis in transgenic Big Blue rats exposed by inhalation to CM44 (similar to "C" fibres) (Bottin et al., 2003).

DNA damage

Using a comet assay, Cavallo et al. (2004) found slight direct and oxidative DNA damage in cultured MeT-5A mesothelial cells exposed to a glass wool sample. Nevertheless, the enhancement was not significant. (Note: It is, however, apparent from the figures that the cell response does not greatly differ from that of other samples—for instance, Danish rock wool.)

One study focused on DNA damage in mesothelial cells following exposure of rats to MMVF11. A significant enhancement of 8-OH-dG in mesothelial cell DNA was detected

10 days postexposure with the lowest doses tested and decreased after 20 weeks. Nevertheless, at 20 weeks, a dose–response increase in 8-OH-dG formation was found (Schürkes et al., 2004).

Mutation
No mutation has been found in transgenic Big Blue rats exposed to CM44 (similar to "C" fibres) (Bottin et al., 2003).

Inflammation
Inoculation of MMVF11 in the peritoneal cavity of rats resulted in a significant macrophage influx 10–20 weeks' postexposure and significant TNF-α release (Schürkes et al., 2004).

In their comparative study of the response of hamsters and rats to MMVF10a, Bermudez et al. (2003) found minimal pleural inflammation in both species. An increase in the number of macrophages and lymphocytes was found in hamsters, and an increase in the number of macrophages was observed in rats.

No increases in BAL parameters, such as polymorphonuclear leukocyte (PMN) influx, release of LDH and proteins, and level of gamma-glutamyl transferase, were observed in rats exposed nose-only to glass fibre type B-01/09 (Creutzenberg et al., 1997).

ROS production
In an in vitro assay investigating the depletion of antioxidants, reduced glutathione (GSH) and ascorbate, Brown et al. (2000) observed ROS production with MMVF10. Similar results were obtained with code 104/475, at an equivalent number of fibres.

Using cell systems, ROS production has been investigated with RAW264.7 and J774 cells exposed to a glass wool sample (Nishiike et al., 2005). The authors found production of nitroxide derivatives, but no oxygen radical generation.

Another cell type, differentiated HL-60-M, was found to produce ROS, as detected by chemiluminescence when exposed to glass wool code A. Fibre preincubation in unbuffered physiological saline resulted in a decrease of this production (Zoller & Zeller, 2000).

Carcinogenicity studies
Alveolar fibrosis and bronchoalveolar hyperplasia were observed in rats exposed to 104E fibres and, at a lower rate, to 104/475 fibres in an inhalation study (Cullen et al., 2000). Lung tumours and pleural mesotheliomas were found following exposure to 104E fibres, and only lung tumours were detected after exposure to 104/475 fibres. It can be noted that at the end of exposure time (12 months), the number of fibres >15 μm in length was higher with the most carcinogenic fibre, 104E, in comparison with 104/475. This contrasted with data after 12 months of recovery, as the opposite was found, suggesting that this time is not the best indicator to correlate with the carcinogenic potency. According to the authors, 104E and 104/475 fibres had similar k_{dis} (dissolution rate constant) values but different chemical stabilities in the lung. This property could account for the observed changes in the relative number of fibres with exposure conditions. In the same study, intraperitoneal inoculation of 10^9 fibres resulted in 87.5% and 33.3% mesotheliomas with 104E and 104/475 fibres, respectively.

A glass fibre type has been found to be carcinogenic in rats after intrapleural inoculation of 20 mg of fibres (Fukada et al., 1987).

Mineral wool (rock wool, slag wool)

Transport of fibres

MMVF21 fibres showed a clearance half-time of 92 days (WHO fibres) following nose-only exposure of rats (Creutzenberg et al., 1997). The clearance half-time, calculated from measurements with tracer particles of $^{46}Sc_2O_3$, showed an enhancement when compared with controls (102 versus 66 days).

Using the same assay, clearance of labelled particles did not significantly differ from that of controls in rats exposed to high-temperature insulation wools: CMS, a calcium magnesium silicate, and CMZS, a calcium magnesium zirconium silicate. Half-times of fibres were approximately 50 and 90 days (WHO fibres) and 10 and 50 days (fibres >20 µm in length) for CMS and CMZS, respectively (Bellmann et al., 2002). In this study, the amount of fibres was measured in the lung and in the lymph nodes. Numbers of fibres found in the lung 3 months after the end of exposure were of the same level (10×10^6 WHO fibres per lung), but more CMS fibres were detected in the lymph nodes. Non-fibrous particles were 0.5 mg/lung.

Phagocytosis

Internalization of two rock wool samples by MeT-5A mesothelial cells in culture was reported by Cavallo et al. (2004).

MMVF21 fibres were reported to be phagocytized by rat and hamster alveolar macrophages (Droger et al., 2000). The median size of the internalized fibres was 7.7 µm in both rat and hamster macrophages. Cytotoxicity resulted in rat macrophages but not in hamster macrophages.

Different samples of stone wools (A, B1, B2 and C) were phagocytized by differentiated U937 cells (Dika Ngua et al., 2005).

Electron microscopic studies revealed that rock wool fibres were phagocytized by rat alveolar macrophages in culture (Kudo et al., 2003).

DNA damage

With their comet assay, Cavallo et al. (2004) found slight direct DNA damage in cultured MeT-5A mesothelial cells exposed to a rock wool sample and a slight direct and oxidative enhancement with a Danish rock wool sample. (Note: It is, however, apparent from the figures that the cell response does not greatly differ from that of other samples.)

Growth alteration

Neither of the high-temperature insulation wools, CMS and CMZS, induced cell proliferation in the terminal airways by inhalation in rats (Brown et al., 2002).

Inflammation

BAL parameters (PMN influx, release of LDH and proteins, and level of gamma-glutamyl transferase) were increased in rats exposed nose-only to MMVF21 (Creutzenberg et al., 1997).

The high-temperature insulation wools, CMS and CMZS, did not modify BAL parameters (cell influx and total proteins) by inhalation in rats (Brown et al., 2002).

Apoptosis
A rock wool sample did not produce DNA fragmentation in cultures of alveolar macrophages from Fischer rats (Kudo et al., 2003).

Apoptosis was found in U937 cells treated with different samples of stone wools (A, B1, B2 and C). The enhancement was significant with the A sample, compared with controls (Dika Ngua et al., 2005).

ROS production
ROS production has been investigated with RAW264.7 and J774 cells exposed to a rock wool sample (Nishiike et al., 2005). The authors found oxygen radical generation and no production of nitroxide derivatives.

HL-60-M cells were found to produce ROS detected by chemiluminescence when exposed to stone wool code G, HT-N and MMVF21. Fibre preincubation in unbuffered physiological saline resulted in an enhancement of this production, except with stone wool code G (Zoller & Zeller, 2000).

Reduction of oxygen consumption, an effect resulting from cytotoxicity, was reported by Kim et al. (2001) in rat alveolar macrophages exposed to rock wool fibres.

Carcinogenicity studies
One rock wool sample, D6 (equivalent to MMVF21), has produced 56% mesotheliomas in rats exposed by intraperitoneal inoculation to 0.5×10^9 WHO fibres (no tumours in control rats). In contrast, no tumours were found in rats exposed to HT Roxul® (MMVF34) (Kamstrup et al., 2002).

Ceramic fibres

Transport of fibres
RCF1 sample has been used in several experiments. Creutzenberg et al. (1997) examined the half-time of tracer-labelled particles in rats. The half-time was found to increase from 66 days in clean air controls to 1200 days in RCF-exposed animals 93 days after a short exposure (3 months). The corresponding half-time for fibres was 113 days.

An RCF sample (equivalent to RCF3) exhibited a high enhancement of tracer half-time and a fibre half-time of about 320 days (WHO fibres) and 15 days (fibres >20 µm in length) (Bellmann et al., 2002).

A comparison between RCF1 and RCF1a samples was carried out in rats (Bellmann et al., 2001). Short-term exposure resulted in greater effects with the RCF1 sample (Brown et al., 2005). The aerosol concentration of fibres >20 µm in length was similar between the two samples (130 fibres/ml); nevertheless, the concentration of WHO fibres and of all fibres was greater in the RCF1 sample compared with RCF1a (679 ± 149 versus 481 ± 162 for WHO fibres). The difference in effects between the two samples does not seem to be related to different half-times of the fibres >20 µm in length, as they were similar; moreover, the lung

burden of these fibres was even lower in the RCF1a sample (Bellmann et al., 2001; Brown et al., 2005). In contrast, clearance half-times of fibres were enhanced and lung burden was greater in animals exposed to RCF1 when other size ranges of fibres were considered. Clearance impairment is assumed to result from the presence of a high content of non-fibrous particles in this sample, in comparison to RCF1a (Brown et al., 2005). Accordingly, retardation of macrophage-mediated clearance of labelled particles was observed in animals exposed to RCF1. (Note: Data on clearance were obtained from two different experiments.) (It may be discussed whether clearance is preferentially retarded in the RCF1 sample for "macrophage fibres" in comparison with long fibres, as for each group of fibres [>20 μm; WHO and all fibres], the half-time increase was similar, about 20%, as compared to RCF1a.)

Histological analyses showed very slight fibrosis with both samples and more inflammatory cells with the RCF1 sample.

Phagocytosis
Internalization of an RCF sample by MeT-5A mesothelial cells in culture was reported by Cavallo et al. (2004).

Alveolar macrophages of rats exposed by inhalation for 2 weeks to a ceramic sample demonstrated an increased phagocytic activity towards heat-inactivated yeast at the end of the exposure time up to 4 weeks of recovery time (Morimoto et al., 1994).

DNA damage
Direct DNA damage in MeT-5A mesothelial cells exposed to an RCF sample and no oxidative DNA damage were found with the comet assay (Cavallo et al., 2004). (Note: It seems that only the lower concentration produced significant enhancement. However, it is apparent from the figures that the cell response does not greatly differ from that of other samples.)

Growth alteration
A cell proliferation in the terminal airways was detected in rats exposed by inhalation to RCF (equivalent to RCF3) (Brown et al., 2002).

Chromosome damage
Chromosome breakage and hyperploidy were detected in cultures of human amniotic fluid cells exposed to an RCF sample (Dopp et al., 1997).

Transformation
RCF1, RCF3 and RCF4 samples produced transformation of SHE cells. RCF3 was the most active, followed by RCF1 and RCF4, which was weakly active. Iron coating of RCF1 and RCF3 attenuated the level of transformation, but the low transforming potency of RCF4 was slightly affected (Elias et al., 2002).

Inflammation
In rats exposed by inhalation, RCF1 fibres produced an increase in BAL cell influx and biochemical parameters (Creutzenberg et al., 1997), and RCF (equivalent to RCF3) produced a slight increase in cell influx and proteins (Brown et al., 2002).

No mRNA expression of cytokines (TNF-α and IL-6), but enhancement of transforming growth factor-beta (TGF-β) expression was detected in lungs of rats, 1 year after exposure to ceramic fibres (Morimoto et al., 2001).

ROS production
In an in vitro assay investigating the depletion of antioxidants, GSH and ascorbate, ROS production was detected with the RCF1 and RCF4 samples (Brown et al., 2000).

Using another type of in vitro assay, Elias et al. (2002) did not find ROS production with RCF1, RCF3 or RCF4 samples; a faint electron paramagnetic resonance (EPR) spectrum was observed in the presence of hydrogen peroxide. In this study, catalytic decomposition of hydrogen peroxide by the iron-coated RCF samples was found.

ROS production, based on the levels of GSH and oxidized glutathione (GSSG), and no oxygen radical generation were found when RAW264.7 and J774 cells were exposed to RCF1 (Nishiike et al., 2005). In this study, production of nitroxide derivatives was enhanced.

Reduction of oxygen consumption, an effect resulting from cytotoxicity, was reported by Kim et al. (2001) in rat alveolar macrophages exposed to ceramic fibres.

Short-term experiment
Histological analyses of lungs of rats exposed to RCF1 and RCF1a samples showed very slight fibrosis with both samples and more inflammatory cells with the RCF1 sample (Bellmann et al., 2001).

Carcinogenicity studies
Based on retention and clearance data, reviewed in Mast et al. (2000) for an RCF1 sample, several authors consider that the tumour rate observed with the highest dose (30 mg/m^3) likely resulted from an overload effect.

Summary on the determinants of carcinogenic potency

The major determinants of carcinogenicity are biopersistence, fibre dimensions and physicochemical properties. The available epidemiological data are not informative for this decision, but they are also not inconsistent. In each class (fibrous, wools, ceramic), there is a range of characteristics. This cannot be summarized in a table as, depending on the characteristics, the potential for carcinogenicity will vary. The working group did not feel comfortable making the same type of evalution.

References

[References being compiled]

2.2.12.4 Physicochemical properties and biopersistence

Iron (trace amounts to 9%) is part of the composition of glass fibres. Their diameter is rather small, but wools have a wide diameter range. Free radical release occurs. Biopersistence is low for glass wool, higher for rock wool and much higher for RCF.

No information is available for mineral wools and ceramic fibres.

2.2.13 **Wollastonite** (Table 2.15)

2.2.13.1 Epidemiological studies

In the only available small cohort mortality study of workers in a wollastonite quarry, the observed numbers of deaths from all cancers combined and lung cancer were lower than expected. A case of a rare malignant mesenchymal tumour in the retroperitoneum of a 73-year-old woman was noted to have features similar to a mesothelioma. Pleural plaques were detected in nine (18%) workers, and four of these lacked any indication of asbestos exposure.

2.2.13.2 Animal studies

Carcinogenicity by inhalation

No tumours were observed in rats exposed to up to 55 WHO fibres/ml, corresponding to 10 mg/m^3. The concentration of WHO fibres in the study was thus low. The exposure duration was 20 months. The follow-up was extended to the natural death of the animals.

Carcinogenicity by intraperitoneal injection

When rats were injected with wollastonite intraperitoneally, no abdominal tumours were found (Pott et al., 1987, 1989; IARC, 1997). Similarly, in another intraperitoneal injection study (Rittinghausen et al., 1991, 1992; IARC, 1997), in which rats were given 30 mg wollastonite, no abdominal tumours were observed. The animals were killed 130 weeks after the start of the treatment.

Additional negative intraperitoneal studies exist (IARC, 1997).

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

No fibrosis was observed in the inhalation carcinogenicity study after 1 year (0/6 studied); after 2 years, 1/6 animals showed interstitial fibrosis.

Proliferation

Intraperitoneal injection of wollastonite induced an early (inflammatory and) proliferative response (5-bromo-2-deoxyuridine [BrdU] incorporation) in mice, which subsided in 21 days (McDonald et al., 1997). Two per cent of the fibres were left after 6 months.

2.2.13.3 Mechanistic data

Clearance and changes in situ of the deposited fibres/biopersistence

Warheit et al. (1999) reported on a study in which rats were exposed for 5 days to aerosols of wollastonite fibres (800 fibres/cm^3, 115 mg/m^3). The wollastonite fibres cleared from the lung rapidly, with a clearance half-life of less than 1 week. During 1 month in the lung, wollastonite mean fibre length progressively decreased from 11 to 6 μm.

Modes of action of fibres in pulmonary/pleural carcinogenesis

No data are available.

Genotoxicity

No data are available.

Malignant transformation, alterations in growth kinetics, inhibition of differentiation

No data are available.

Cell proliferation

No data are available.

Chronic inflammation, release of cytokines, growth factors, reactive species

Pulmonary toxicity of wollastonite has been studied by Tatrai et al. (2004) using both in vivo long-term sequential and in vitro methods in Sprague-Dawley rats. By the end of 6 months, wollastonite had induced mild pulmonary interstitial fibrosis, whereas crocidolite induced progressive interstitial fibrosis as a function of time. Wollastonite decreased the activity of gamma-glutamyl transpeptidase and glutathione peroxidase and increased expression of proinflammatory peptides. The effects caused by wollastonite were mild compared with those of crocidolite.

Short-term animal tests

No data are available.

Carcinogenicity studies

Pott et al. (1987, 1989) showed that intraperitoneal injection of 0.5 mg wollastonite did not induce lung tumours in rats.

Summary on the determinants of carcinogenic potency

It seems that wollastonite induces pulmonary interstitial fibrosis in rats, but not lung tumours.

References

[References being compiled]

2.2.13.4 Physicochemical properties and biopersistence

Data on free radical release are ambiguous. Fibres are <1 μm in diameter and >5 μm in length.

Biopersistence is low, but there is an indication from the IARC Monograph that wollasonite may be contaminated with carcinogenic minerals. Biopersistence is low without contaminants and medium with contaminants.

2.2.14 Xonotlite (Table 2.16)

2.2.14.1 Epidemiological studies

No data are available.

2.2.14.2 Animal studies

Carcinogenicity by inhalation

No data are available.

Carcinogenicity by intraperitoneal/intrapleural injection

Calcium silicate (Syn-Xo-1) at a dose of 1 mg did not induce intrapleural tumours in rats after intrapleural administration in a 25-month study; in this study, two chrysotile samples (of five) and one sepiolite sample (of two) induced mesotheliomas (Fukuda et al., 1985–87).

Fibrosis in inhalation exposure/intratracheal instillation/intraperitoneal injection

No fibrosis occurred after intratracheal instillation of 10 mg or 5 mg (Lemaire et al., 1989). Calcium silicate (Syn-Xo-1) did not induce fibrosis in rats (25-month experiment) or mice (18-month experiment) after intraperitoneal administration.

Proliferation

No data are available.

2.2.14.3 Mechanistic data

Xonotlite is negative in most test systems: there is no oxidative DNA damage, and it did not induce free radicals. There was a positive result in Chinese hamster ovary cells.

2.2.14.4 Physicochemical properties and biopersistence

Xonotlite fibres are small in diameter (<1 μm) and length (<2 μm). They are very soluble. Xonotlite does not induce free radicals.

2.3 Summary consensus report[1]

2.3.1 Introduction

The WHO Workshop on Mechanisms of Fibre Carcinogenesis and Assessment of Chrysotile Asbestos Substitutes was convened at IARC in Lyon in response to a request from the Intergovernmental Negotiating Committee (INC) for the Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade (see page XX). The substitutes considered by the WHO workshop included the 12 chrysotile substitutes identified by the INC for priority assessment by WHO, 2 substances from a second list provided by the INC to be assessed if resources allow and 1 further substance for which data were submitted in response to WHO's public "call for data" for the workshop.

The workshop opened on 8 November with a one-day session devoted to taking statements from observers mainly representing various commercial interests, along with some government observers and the Rotterdam Convention Secretariat. In addition, a statement submitted by a labour organization was read by the workshop Secretariat. Observers were invited to submit any comments on the pre-workshop working drafts in writing. Invited specialists and observers did not participate in the evaluations of the substitutes (see section 2.3.3) or the final agreement of Part 1 (see section 2.3.2). A list of participants appears on page XX.

2.3.2 Part 1: Methodological aspects

The workshop considered the mode(s) of action of fibre carcinogenesis and the developments in the field after the IARC (1996) report, but did not produce a formal assessment of the state of the art. The workshop established a framework for hazard assessment based on epidemiological data (whether data are sufficient to determine carcinogenicity); in vivo animal data (whether there is an indication of carcinogenicity or lung fibrosis); mechanistic information (whether critical indicators of carcinogenicity exist, e.g. positive results for genotoxicity in in vitro tests); and physicochemical and biopersistence data as determinants of dose at the target site and possible indicators of carcinogenic potential. The workshop conclusions on each of these factors appear in the following paragraphs.

In light of the workshop scope to assess fibrous forms of the substitutes, the workshop confined its considerations to effects related to cancer, focusing on lung cancer, mesothelioma and lung fibrosis. Further, noting that substitutes may be used in a variety of applications with different exposure potential, either alone or in combination with other substances, the workshop did not embark on risk assessment, but rather limited its work to assessing the hazard.

Epidemiological studies on fibres have a clear advantage over toxicological studies in that they involve studies of humans. They also have the advantage that they study the

[1] This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

effects of exposure in the real world where the effects of these exposures may be mitigated or enhanced by other factors. Despite these obvious advantages, the presence or absence of evidence of risk from epidemiological studies does not always override contrary findings from toxicological studies. The interpretation of either positive or non-positive epidemiological findings needs to be carefully considered in light of the strengths and weaknesses of the study design.

In in vivo animal studies, carcinogenic response (lung cancer, mesothelioma) and fibrosis were considered to be the key effects; epithelial cell proliferation and inflammation were not regarded to be equally important indicators of human health hazard. From studies with asbestos, it is apparent that the sensitivity of the rat to fibre-induced lung tumours in inhalation studies is clearly lower than that of humans. This holds true when the effect is related to exposure concentrations and lung burdens. The workshop discussed the hypothesis that differences in sensitivity could be due to greater lung mass and/or longer lifespan in humans; however, the question remains open as to whether this sensitivity difference remains if individual rat or human lung cells are taken as a basis for comparison. In comparison, testing of fibres by intraperitoneal injection represents a useful and sensitive assay, which also avoids confounding effects of granular dusts.

Genotoxic potential in experimental systems can be assessed via cell-free in vitro assays, in vitro tests with cultured cells and in vivo studies, usually in mice or rats. Fibres may act in principle on all steps in tumour development. However, of these interactions, the in vitro genotoxicity tests are mainly indicative of genotoxic effects involved in the first steps of tumour initiation. Effects related to biopersistence of fibres (such as continuous “frustrated phagocytosis”) and secondary genotoxicity arising from ROS and RNS and mitogen release by macrophages and inflammatory cells are not detected in routinely used genotoxicity tests. Therefore, negative results indicate a lack of primary genotoxicity, but do not exclude effects on later steps of carcinogenesis. A completely inert fibre that could be used as a negative control in the above-mentioned assays has not been identified.

The chemical composition of the substitutes is a key factor influencing structure and physicochemical properties, such as surface area, surface reactivity and solubility. Attention should be paid not only to the chemical composition of the fibres, including their major and trace elements, but also to contaminants or accompanying elements, including their speciation. Fibre-derived free radical generation favours DNA damage and mutations. Surface properties are a determining factor in the inflammatory response. In relation to fibre dimension and deposition, one can assume that there exists a continuous variation in the carcinogenic potency of respirable fibres, which increases with length. Biopersistence of a fibre increases tissue burden and therefore may increase any toxicity the fibre might possess. For synthetic vitreous fibres, there is evidence in animals that the potential for carcinogenicity increases with biopersistence. This has not been demonstrated, however, for other fibres.

For all fibres, the fibres must be respirable to pose an appreciable hazard. Respirability is mainly determined by diameter and density; thus, with a given fibre diameter, a higher specific density is associated with lower respirability (note that the specific density of most organic fibres is lower than the specific density of inorganic fibres).

2.3.3 Part 2: Hazard assessment

The workshop decided to group substitutes roughly into hazard groupings of high, medium and low. However, for some substitutes, there was insufficient information to draw any conclusion on hazard, and in this case the workshop categorized the hazard as indeterminate (a category that is not comparable to the other groupings). The hazard groups high, medium and low should be considered in relation to each other and did not have reference to formal criteria or definitions, as such. For details of each substance, the reader is referred to section 2.2. It is important to note that for each substitute, the fibre dimensions of commercially available products may vary, and the workshop did not assess this variation. The substitutes are listed below in alphabetical order.

***para*-Aramid** releases respirable fibres with dimensions similar to those of known carcinogenic fibres. *p*-Aramid fibres have induced pulmonary effects in animal inhalation studies. Biopersistence was noted. The workshop considered the human health hazard to be **medium**.

Most natural deposits contain **attapulgite** fibres that are <5 μm in length, and at workplaces the mean fibre length was <0.4 μm. The hazard from exposure to respirable attapulgite is likely to be **high for long fibres** and **low for short fibres**. This assessment is mainly based on findings in long-term inhalation experiments in animals, in which tumours were seen with long fibres; no tumours were seen in studies with short fibres.

The nominal diameter of **carbon fibres** ranges from 5 to 15 μm. Workplace exposure in production and processing is mostly to non-respirable fibres. The workshop considered the hazard from inhalation exposure to these fibres to be **low**.

Most **cellulose fibres** are not respirable; for these, the hazard is **low**. For respirable fibres, the available data do not allow the evaluation of the hazard; the hazard is thus **indeterminate**.

The dimensions of **graphite whiskers** indicate high respirability, and they have a long half-time in the lungs. However, in the absence of any further useful information, the hazard from inhalation exposure was considered to be **indeterminate**.

Magnesium sulfate whiskers did not induce tumours in limited inhalation and intratracheal administration studies, were negative in limited short-term tests and are very quickly eliminated from the lung. It was discussed whether the hazard grouping should be **low** or **indeterminate**. On the basis of the data available, in the time available, consensus was not reached.

For respirable **polyethylene**, **polyvinyl chloride** and **polyvinyl alcohol fibres**, the data were insufficient for hazard classification, and the working group thus considered the hazard **indeterminate**.

In facilities producing **polypropylene fibres**, exposure to respirable fibres occurs. After intratracheal administration, respirable polypropylene fibres were highly biopersistent; however, no fibrosis was reported in a subchronic animal study. However, the data are sparse, and the human health hazard potential was considered to be **indeterminate**.

The workshop considered that respirable **potassium octatitanate fibres** are likely to pose a **high** hazard to humans after inhalation exposure. At workplaces, there is exposure to respirable fibres. There was a high and partly dose-dependent incidence of mesothelioma after intraperitoneal injection in two species (high incidence indicating high potency). There is evidence of genotoxicity. Biopersistence was noted.

Wool-like **synthetic vitreous fibres** (including glass wool/fibrous glass, mineral wool, special-purpose vitreous silicates and RCF) contain respirable fibres. For these fibres, the major determinants of hazard are biopersistence, fibre dimensions and physicochemical properties. It was noted that the available epidemiological data are not informative, due to mixed (vitreous fibre) exposures or other design limitations. Based on inhalation exposure studies, intraperitoneal injection studies and biopersistence studies, it was concluded that the carcinogenic hazard could vary from high to low, with **high** for the biopersistent fibres and **low** for non-biopersistent fibres.

Natural **wollastonite** contains respirable fibres. In occupational settings, exposure is mainly to short fibres. In chronic studies, wollastonite did not induce tumours after intraperitoneal injection in animals; however, samples of wollastonite were active in different studies for genotoxicity. After considering this apparent discrepancy, it was concluded that the hazard was likely to be **low.**

In a limited study with intraperitoneal implantation, **xonotlite** did not induce tumours. After intratracheal injection in a chronic study, no inflammatory or fibrotic reaction of the lung was observed. The chemical composition of xonotlite is similar to that of wollastonite, but it is more rapidly eliminated from the lung. The workshop considered the human health hazard to be **low.**

Table 2.1 Physicochemical properties, biopersistence data and mechanistic data for aramid and *para*-aramid

End-point	Fibre modification	Experimental condition	Results	References
Fibre dimensions				
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence	*p*-Aramid RFP	Exposure of rats	Rapid clearance of fibres in the lung	Warheit et al. (1992)
		Exposure of rats	Rapid clearance of fibres in the lung	Warheit et al. (1996)
		Inhalation studies in hamsters and rats	*p*-Aramid RFP are biodegradable in the lungs	Warheit et al. (1997)
		Inhalation study in rats for 5 days (900–1344 fibres/cm^3, 9–11 mg/m^3)	Rapid clearance from the lung (half-life: 30 days)	Warheit et al. (1999)
		Instillation into rat lungs	Shortening of fibres	Warheit et al. (2002)
		Subchronic and chronic inhalation exposure of rats	Shortening of fibres and clearance from the lung	Kelly et al. (1993)
		Inhalation exposure of rats	Shortening of fibres	Searl (1997)
		Subchronic exposure of rats	Shortening of fibres	Bellmann et al. (2000)
		In vivo and in vitro studies	Fibres are biodegradable in the lungs	Warheit et al. (2005)
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis	*p*-Aramid RFP	Exposure of rats	Cystic keratinizing lung lesions were observed	Warheit (1995)
		Inhalation study in rats (2 years)	Cystic keratinizing lung lesions were observed	Frame et al. (1997)

End-point	Fibre modification	Experimental condition	Results	References
Genotoxicity	*p*-Aramid RFP	Chromosomal aberrations in cultured human peripheral blood lymphocytes (fibre concentrations: 6–400 µg/ml; exposure time: 19 h)	No significant increase of chromosomal aberrations, polyploidy or endoreduplication	Warheit et al. (2001)
Malignant transformation, alterations in growth kinetics, inhibition of differentiation	Kevlar aramid	Proliferative capacity, CFE and ODC activity were measured in THE cells and in rat lung fibroblasts (RL90)	In HTE cells, aramid caused a significant increase in [^{3}H]thymidine incorporation and CFE and produced a dose-dependent induction of ODC enzyme activity. Proliferative effects by aramid were not observed in RL90 fibroblasts.	Marsh et al. (1994)
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species		Rats were exposed for 2 weeks to aerosols of *p*-aramid fibrils (750 fibres/cm^3)	Transient pulmonary inflammatory and cell labelling responses in terminal bronchiolar and subpleural regions were observed	Warheit et al. (1996)
Short-term animal tests	Ultrafine Kevlar fibrils	Inhalation experiment in rats	Cystic keratinizing squamous cell carcinoma was found Experimentally induced lung tumours in rats	Lee et al. (1988)
	p-Aramid RFP	Subchronic exposure of rats	Shortening of fibres	Bellmann et al. (2000)

Table 2.2 Physicochemical properties, biopersistence data and mechanistic data for attapulgite

	Attapulgite	References
Fibre dimensions	Fibre mean diameter: 0.06 µm **Fibre mean length: 1 µm; range = 0–5 µm (attapulgite from the deposits of Leicester, UK, and Toregon, Spain, length >5 µm)**	Bignon et al. (1980); IARC (1997)
Chemical composition	$(Mg, Al)_2Si_4O_{10}(OH)\cdot 4H_2O$ natural aluminium magnesium silicate with magnesium partially replaced by aluminium or iron (Fe^{2+}, Fe^{3+}) Chemical analysis: SiO_2 60–61%; Al_2O_3 10–11%; MgO 10.2%; CaO 2%; ? 0.14–0.15%; Fe_2O_3 3%; FeO 0.4%; TiO_2 0.5%	Clark et al. (1990); IARC (1997)

Tumour induction	Experimental	Fibres	Results	References
	In vitro study: measure modification of cell growth Cells: rat pleural mesothelial cells Fibre concentrations: 2–10 µg/cm^2 Fibres tested: chrysotile Rhodesian UICC (Ch A) and short Canadian fibres (Ch C), attapulgite	Fibre mean length = 0.77 µm Fibre mean diameter = 0.06 µm	Attapulgite and Ch C did not modify cell growth except at high doses (10 µg/cm^2)	Renier et al. (1989)
	Intraperitoneal injection Animals: Wistar rats No. of animals: 40 Dose: 25 mg/animal	Attapulgite origin unspecified 30% of fibres >5 µm in length	77% of rats treated developed malignant tumours of the abdominal cavity	Pott et al. (1987)
	Intraperitoneal injection and intratracheal instillation study Animals: rats Dose: 60 mg/animal Fibres tested: about 50 dusts	Attapulgite from three sources with short fibre lengths and an attapulgite sample with long fibres	Attapulgite with short fibre lengths was not shown to be carcinogenic, but an attapulgite sample with longer fibres had a moderate effect	Pott et al. (1987)

Tumour induction	Experimental	Fibres	Results	References
	Intrapleural injection study Animals: Sprague-Dawley rats No. of animals: 20 for each treatment Dose: 20 mg/animal Fibres tested: chrysotile, amosite, crocidolite, attapulgite	Two samples from Spain: fibre mean length <2 µm (diameter unspecified) One sample from Leicester, UK: fibre mean length >6 µm, diameter <0.5 µm	Rats exposed to attapulgite from Spain: 2/40 rats had hyperplasia, 1/40 had peritoneal mesothelioma Rats exposed to attapulgite from Leicester: 30/32 rats had pleural mesothelioma	Wagner et al. (1987)
	Intrapleural injection study Animals: Fischer 344 rats (male) No. of animals: 20 Dose: 20 mg/animal	Fibre mean length = 0.77 µm Fibre mean diameter = 0.06 µm	Attapulgite fibres did not induce tumours, and the mean survival time was of the same order as that observed in the control groups	Jaurand et al. (1987)
	Intrapleural injection study Animals: Sprague-Dawley rats Dose: 20 mg/animal Fibres tested: chrysotile Rhodesian UICC (Ch A) and short Canadian fibres (Ch C), attapulgite	Fibre mean length = 0.77 µm Fibre mean diameter = 0.06 µm	The incidence rates of mesothelioma were 0% (saline controls), 0% (attapulgite), 19% (ChC) and 48% (Ch A)	Renier et al. (1989)
	Intrapleural injection study Animals: Fischer 344 rats No. of animals: 25 for each treatment Doses: 0.5, 2, 4, 8, 16 or 32 mg/animal Fibres tested: attapulgite, erionite	Attapulgite from USA: fibre mean length <1 µm diameter <0.1 µm	2/140 rats treated with attapulgite and 137/144 rats treated with erionite had mesothelioma Incidence in the control group 1/79	Coffin et al. (1992)

Tumour induction	Experimental	Fibres	Results	References
	Long-term inhalation assay Animals: Fischer 344 rats (male) No. of animals: 20 Time of exposure: 12 months (6 h/day, 5 days/week) Exposure concentration: 10 mg/m^3	Two samples were studied: sample from Spain: fibre mean length <2 µm (diameter unspecified) sample from Leicester, UK: fibre mean length >6 µm, diameter <0.5 µm	Rats exposed to attapulgite from Spain: 3/40 rats had hyperplasia, 1/40 had peritoneal mesothelioma Rats exposed to attapulgite from Leicester: 8/40 rats had hyperplasia, 2/40 had benign alveolar tumours, 1/40 had malignant alveolar tumours, 3/40 had mesothelioma	Wagner et al. (1987)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Intratracheal lobe exposition study Animals: sheep No. of animals: 16 Dose: 100 mg/sheep Fibres tested: UICC asbestos, attapulgite	Sample from Florida Mean fibre length = 0.8 µm	Attapulgite increases the total BAL cells (macrophages, neutrophils, fibronectin), lactate dehydrogenase (LDH) and β-glucuronidase BAL cellularity returns to control levels by 60 days, whereas in the UICC asbestos–exposed sheep, it remained significantly above control Macrophagic alveolitis with minimal airway distortion in all attapulgite-exposed sheep was observed (marked alveolitis in all asbestos-exposed sheep)	Bégin et al. (1987)
	Intratracheal instillation Animals: rats No. of animals: 5 for each treatment Doses: 1, 5 and 10 mg/rat Fibres tested: UICC chrysotile B, short chrysotile 4T30, attapulgite, xonotlite and Fiberfrax (an aluminium silicate)	Fibre mean length = 0.8 µm (100% <1 µm) Fibre mean diameter = 0.1 µm Specific surface area = 133 g/m^2	One month later, attapulgite, at doses up to 10 mg, caused minimal reactions characterized by mononuclear cell infiltration mainly in the alveolar structures Lung biological reactivity observed for the various silicates was xonotlite << attapulgite < short chrysotile 4T30 < Fiberfrax < UICC chrysotile B	Lemaire et al. (1989)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Transtracheal injection study Animals: rats No. of animals: 5 for each treatment Fibres tested: UICC chrysotile B asbestos, short chrysotile 4T30, attapulgite, xonotlite and Fiberfrax (an aluminium silicate)	Fibre mean length = 0.8 µm (100% <1 µm) Fibre mean diameter = 0.1 µm Specific surface area = 133 g/m^2	One month later, granuloma formation (in rats treated with attapulgite and short chrysotile) or fibrosis (UICC chrysotile B) is observed Enhanced production of interleukin-1 (IL-1)-like activity was seen during the early stage (1 month) and decreased thereafter (8 months) After 8 months, the granulomatous reactions had resolved or greatly diminished	Lemaire (1991)
	Long-term inhalation assay Animals: Fischer 344 rats (male) No. of animals: 20 Time of exposure: 12 months (6 h/day, 5 days/week) Exposure concentration: 10 mg/m^3	Two samples were studied: sample from Spain: fibre mean length <2 µm (diameter unspecified) sample from Leicester, UK: fibre mean length >6 µm, diameter <0.5 µm	Rats exposed to attapulgite from Spain: score of fibrosis = 3.2 Rats exposed to attapulgite from Leicester: score of fibrosis = 4.0	Wagner et al. (1987)
	Short-term inhalation assay Animals: Fischer 344 rats (male) No. of animals: 20 Time of exposure: 12 months (6 h/day, 5 days/week) Exposure concentration: 10 mg/m^3 Fibres tested: paligorskyte, UICC crocidolite	Two milled samples were studied: sample from Spain: fibre mean length <2 µm, 0.54% of fibres >6 µm in length (diameter unspecified) sample from Leicester, UK: 20% of fibres >6 µm in length, diameter <0.5 µm	The paligorskyte produced fibrosis similar to or more severe than those produced by UICC crocidolite	Wagner et al. (1987)

Cytotoxicity	Experimental	Fibres	Results	References
		One Spanish attapulgite sample and three samples of drugs sold in France	The haemolytic activities of a Spanish attapulgite sample and of three samples of drugs sold in France were greater than or similar to that of UICC chrysotile asbestos.	Bignon et al. (1980)
	In vitro study: measure of LDH release Cells: Swiss mouse peritoneal macrophages Fibre concentration: 150 µg/ml Fibres tested: chrysotile, attapulgite clay, Fiberfrax (an aluminium silicate) and xonotlite	Fibre dimensions were not given	Short-fibre attapulgite caused more release of LDH compared with long-fibre attapulgite	Chamberlain et al. (1982)
	In vitro study: induction of giant cells Cells: human lung carcinoma cells (A549) Fibre concentration: 200 µg/ml	Fibre dimensions were not given	Short fibres did not induce formation of giant cells	Chamberlain et al. (1982)
	In vitro study: cloning efficiency Cells: Chinese hamster lung fibroblast (V79-4)	Fibre dimensions were not given	Short fibres did not modify the cloning efficiency Long fibres reduced (at concentration of 52 µg/ml) the cloning efficiency by 50%	Chamberlain et al. (1982)
	In vitro study: measure of cytotoxic activity Cells: rabbit pulmonary alveolar macrophages and rat pleural mesothelial cells Fibre concentration: 4–10 µg/cm^2		Attapulgite was found to be cytotoxic to rabbit alveolar macrophages at concentrations >4 µg/cm^2 Attapulgite was found to be cytotoxic to rat pleural mesothelial cells at concentrations >10 µg/cm^2	Jaurand et al. (1987)

Cytotoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of membranolytic and cytotoxic activity Cells: rat pulmonary alveolar macrophages Fibre concentrations: 50 and 250 µg/ml Fibres tested: chrysotile, attapulgite clay, Fiberfrax (an aluminium silicate) and xonotlite	Fibre mean length = 0.8 µm (100% <1 µm) Fibre mean diameter = 0.1 µm Specific surface area = 133 g/m^2	At 50 µg, cytotoxic effects were in the decreasing order: Fiberfrax > attapulgite > chrysotile > xonotlite At 250 µg: all four silicates were equivalent Attapulgite was less haemolytic than the xonotlite and Fiberfrax, but was nevertheless highly haemolytic to the rat erythrocytes	Nadeau et al. (1987)

Free radical generation	Experimental	Fibres	Results	References
	In vitro study (cell-derived radicals): fibre-mediated endothelial cell injury study Cells: human umbilical vein and bovine pulmonary artery endothelial cell Fibres tested: amosite and chrysotile asbestos, attapulgite, fibreglass		Attapulgite-induced endothelial cell injury concentration and time dependent, similar to amosite (chrysotile and fibreglass were much less toxic) Superoxide dismutase (SOD), catalase and deferoxamine (iron chelator) produced significant protection, suggesting that iron, via the modified Haber-Weiss reaction, may promote hydroxyl radical formation and contribute to endothelial cell injury induced by these particulates	Garcia et al. (1989)
	I**n vitro study (fibre-derived radicals):** DNA oxidation Fibres tested: crocidolite, attapulgite, glass fibres, potassium titanate, magnesium sulfate whiskers		No increase of 8-hydroxydeoxyguanosine (8-OH-dG) except for crocidolite + $FeSO_4$: increased yield of 8-OH-dG by attapulgite, fibreglass and potassium titanate more than by asbestos	Adachi et al. (1992)

Genotoxicity	Experimental	Fibres	Results	References
	In vitro study: induction of unscheduled DNA synthesis (UDS) Cells: rat hepatocytes in primary culture Fibre concentrations: 2 and 20 µg/cm^2 Fibres tested: attapulgite, xonotlite, sepiolite		None of the fibres showed detectable UDS-eliciting activity	Denizeau et al. (1985)
	In vitro study: induction of sister chromatid exchange Cells: rat pleural mesothelial cells Fibre concentrations: 2 and 4 µg/cm^2 Fibres tested: UICC crocidolite, attapulgite	Sample from Senegal Fibre length <2 µm	Attapulgite did not induce UDS or sister chromatid exchange Crocidolite produced a weak effect	Achard et al. (1987)
	In vitro study: induction of UDS Cells: rat pleural mesothelial cells Fibre concentrations: 2 and 20 µg/cm^2 Fibres tested: chrysotile Rhodesian UICC (Ch A) and short Canadian fibres (Ch C), attapulgite	Fiber mean length = 0.77 µm Fiber mean diameter = 0.06 µm	Attapulgite did not significantly induce UDS at doses tested UDS was, in contrast, stimulated with either Ch A or Ch C	Renier et al. (1989)
	In vitro study: induction of UDS Cells: rat pleural mesothelial cells Fibre concentrations: 2 and 20 µg/cm^2 Fibres tested: chrysotile, crocidolite and attapulgite		Attapulgite did not enhance UDS in contrast to chrysotile and crocidolite	Renier et al. (1990)

Table 2.3 Physicochemical properties, biopersistence data and mechanistic data for carbon fibres

	Carbon fibres	References
Chemical composition	Carbon fibre (amorphous): carbon 90–97%, nitrogen <10%, oxygen ~1%, hydrogen <1% Carbon fibres are further subdivided into PAN based (derived from polyacrylonitrile fibre), pitch based (derived from coal tar or petroleum) or rayon based	
Fibre dimensions	Fibre diameter range = 3–8 μm Fibre median length = 37.5 μm (97% of the fibres were ≤200 μm long) PAN-based carbon fibres are generally too thick to be human respirable, whereas pitch-based carbon fibres are made in smaller diameters that are more likely to be respirable	Zhang et al. (2001)
Dissolution	Insoluble in Gamble's solution No modification at the fibre surface after immersion in saline solutions	IPCS (1993)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Intratracheal instillation assay Animals: rats Fibres tested: chrysotile and carbon fibres	Fibre diameter range = 1–2 μm (30% of fibres with diameters 3–5 μm) Fibre length range = 1–5 μm (30% of the fibres with length 6–10 μm)	Carbon fibres induce less fibrosis than chrysotile	Troitskaia et al. (1984)
	Intratracheal instillation assay Animals: rats Fibre tested: carbon fibres	Two samples: carbon fibres from cellulose and carbon fibres from PAN	Slight fibrosis (measured by BAL evaluation) after 12 months The fibrosis was less for animals exposed to cellulose-derived carbon fibres	Fediakina (1984)
	Short-term inhalation assay Animals: rats Fibre tested: carbon fibres	Two samples: respirable carbon fibres from pitch and carbon fibres from PAN (diameter >9 μm), non-respirable, used as negative control	Pitch-based carbon fibres produced a dose-dependent transient inflammatory response in the lungs of exposed rats (elevated levels of neutrophils, LDH, total protein or alkaline phosphatase in BAL)	Warheit et al. (1995)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Long-term inhalation assay Animals: rats Exposure concentration: 20 mg/m^3 Time of esposure: 16 weeks (6 h/day, 5 days/week) Fibre tested: carbon fibres	Fibre from PAN, non-respirable (mean fibre diameter 7 µm)	No inflammatory or fibrogenic response	Owen et al. (1986)
	Long-term inhalation assay Animals: rats Time of exposure: 16 weeks (6 h/day, 5 days/week) Fibre tested: carbon fibre	Fibre from PAN, non-respirable (mean fibre diameter 3.5 µm)	No inflammatory or fibrogenic response	Waritz et al. (1998)

Cytotoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of cytotoxic activity Cells: rabbit pulmonary alveolar macrophages Dust tested: carbon fibres, quartz and alumina particles	74% of fibres with diameter <3 µm	2/5 of carbon fibre samples were cytotoxic for macrophages	Martin et al. (1989)
	Intratracheal instillation assay Animals: rats Dusts tested: carbon fibres, quartz and alumina particles	74% of fibres with diameter <3 µm	The cytotoxicity increases in the order alumina, carbon fibres, quartz	Martin et al. (1989)

Genotoxicity	Experimental	Fibres	Results	References

Genotoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of cytotoxic activity Cells: rabbit pulmonary alveolar macrophages Dusts tested: carbon fibres, quartz and alumina particles	74% of fibres with diameter <3 µm	2/5 of carbon fibre samples were cytotoxic for macrophages	Martin et al. (1989)

Table 2.4 Physicochemical properties, biopersistence data and mechanistic data for cellulose fibres

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions				
Deposition of inhaled fibres		Study of lung biopsies	Cellulose fibres were detected in 99 of 114 specimens; frequency and deposition were not reported	Pauly et al. (1998)
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence	Cellulose RFP	Inhalation study in rats	Cellulose fibres are more persistent than chrysotile in the rat	Muhle et al. (1997)
		In vivo and in vitro studies	Cellulose fibres are more resistant in lung than *p*-aramid fibres	Warheit et al. (2005)
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis		Injection of high-purity cellulose fibres into the abdominal cavity of rats	Multiple large nodules and widespread adhesions were found in all animals 9 (18%) had malignant tumours (mesothelioma)	Cullen et al. (2002)
Genotoxicity				
Malignant transformation, alterations in growth kinetics, inhibition of differentiation				
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species	Cellulose acetate fibres	Inflammatory response by respiratory burst of neutrophils	Apoptosis was increased 12% with exposure to the more aged fibres versus 2% with the new fibres	Moore et al. (2001)

End-point	Fibre modification	Experimental conditions	Results	References
	Pure cellulose fibres	Toxicity in mouse macrophages in vitro	Release of LDH Release of inflammogenic materials from macrophages Release of similar amounts of plasminogen activator and IL-1 to asbestos Stimulation of release of the inflammogenic agents prostaglandin PGE2 and leucotrine LTC4	Godelaine & Beaufay (1989a,b)
Short-term animal tests	Cellulose dust	Intraperitoneal injection in mice (10^4–10^8 fibres)	Dose-dependent recruitment of inflammatory cells to the mouse peritoneal cavity	Cullen et al. (2000)
		Inhalation in rats	Early inflammatory response in rat lungs	
			In vitro production of TNF-α by lavaged alveolar macrophages was depressed	
	Cellulose dust	Intratracheal exposure of rats (5 mg/kg)	Transient inflammation in the lungs of rats and increased 4-hydroxyproline levels Minimal to mild, non-progressing granulomatous pneumonitis	Morgan et al. (2004)
	Cellulose dust	Cell count, protein, phospholipid, LDH and acid phosphatase were determined in BAL fluid 1, 3 and 7 days after intratracheal instillation of rats	Interstitial oedema as well as the initial signs of inflammation could be detected in the lung after the first day Inflammation after 1 week could be noted	Adamis et al. (1997)
	Cellulose dust	BAL (rats, 15 mg per animal)	Fibrosing granulomatous alveobronchiolitis and an increase of IgA production Pulmonary fibrosis	Tatrai et al. (1996)
	Cellulose dust	Intratracheal injection in hamsters	Fibrosing granulomas	Milton et al. (1990)
	Cellulose fibres from material designed for TLC	Intratracheal injection (15 mg/rat)	Peribroncheolar granulomas in vivo Release of reactive oxygen intermediates in vitro	Tatrai et al. (1992, 1995)
	Aerosol generated from cellulose building insulation	Inhalation study in rats	Alveolitis and epithelial cell hyperplasia	Hadley et al. (1992)

End-point	Fibre modification	Experimental conditions	Results	References
Carcinogenicity studies	Case–control study of 299 malignancies among paperwool workers		Only neoplasms were found	Rinsky (1990)
	Mortality analysis of 1010 deaths of workers in the pulp and paper industry		Excess of cancer, largely lung cancer, but no information on smoking habits	Solet et al. (1989)
	Mortality studies on soft paper mill production unit in Sweden		Mortalities from chronic obstructive pulmonary disease and from asthma were increased No excess in malignancies	Jarvholm et al. (1988)
			Dose-related irritation of the upper respiratory tract	Ericsson et al. (1988)
			Nonspecific reactions to the heavy exposure	Thoren et al. (1989)

Table 2.5 Mechanistic data, physicochemical properties and biopersistence data for graphite whiskers

	Graphite whiskers	References
Chemical composition	Graphite whiskers (crystalline; made by further heating amorphous carbon fibres): carbon 99%, nitrogen, oxygen, hydrogen <??	
Fibre dimensions	Geometric mean diameter = 0.9 µm Geometric mean length = 7 µm	Zhang et al. (2001)
Dissolution	Insoluble in Gamble's solution No modification at the fibre surface after immersion in saline solutions	IPCS (1993)

Biopersistence	Experimental	Fibres	Results	References
	Short-term inhalation assay Animals: Wistar rats Exposure concentration: 151.7 ± 78.8 fibres/ml (8.3 ± 2.2 mg/m^3) Time of exposure: 4 weeks (6 h/day, 5 days/week) Fibre tested: graphite whiskers	Geometric mean diameter = 0.86 µm Geometric mean length = 6.8 µm	The longer whiskers (>20 µm) tended to remain selectively in the lung after the 12-month clearance The whiskers were broken down in the lung The surface of the whiskers peeled away in the lung as a result of their stratified crystalline structure, so thinner and longer whiskers would increase after an extended clearance period The clearance rate tends to decrease with time, which is consistent with the fact that the percentage of graphite whiskers longer than 20 µm increased during the 12-month clearance period	Ishimatsu et al. (2003)

Table 2.6 Physicochemical properties, biopersistence data and mechanistic data for magnesium sulfate whiskers

	Magesium sulfate whiskers	References
Fibre dimensions and aerodynamic diameter	Fibre diameter: 0.35 µm, range 0.30–0.46 µm Fiber mean length: 11.0 µm; range = 0–55 µm	UBE industry; Hori et al. (1994); Fujino et al. (1995)
Chemical composition	$MgSO_4\,5Mg(OH)_{23}\,H_2O$ whisker manufactured by hydrothermal synthesis from magnesium sulfate and magnesium hydroxide Chemical analysis: MgO 66–66.5%; CaO 0.55–0.57%; SiO_2 0.14–0.15%; Fe_2O_3 0.04–0.05%; Al_2O_3 0.04%; B_2O_3 0.21% Metal traces (µg/g): Mn (41); Zn (9); Ni (5); Sr (4); As (0.68); Pb (0.50); Cd (3)	UBE industry
Dissolution	Solubility (37 °C) in water 0.03 g/l; Gamble 0.46 g/l; physiological solution 0.047 g/l	Hori et al. (1994)

Biopersistence	Experimental	Fibres	Results	References
	Short- and long-term inhalation assay Animals: Wistar rats (male) No. of animals: 42 for each study Time of exposure: 4 weeks or 1 year (6 h/day, 5 days/week) Exposure concentration (mg/m^3): 4.0 (short-term assay) and 1.8 (long-term assay)	Two types of magnesium sulfate whiskers of different lengths (long and short) Fibre diameter (SD), µm: long = 0.44 (1.6); short = 0.31 (1.3) Fibre length (SD), µm: long = 12 (2.3); short = 12 (2.1) The whiskers had large aspect ratios, with a few non-fibrous particles	Magnesium sulfate whiskers are dissolved and eliminated rapidly from the lungs (few whiskers detected in the rat lungs even at 1 day after the exposure) Biological half-time (BHT) = 17.6 min	Hori et al. (1994)
	Intratracheal instillation assay Animals: golden hamsters No. of animals: 30 Whiskers concentration: 1.1 mg	The same as the previous experiment	High solubility in lung fluids	Hori et al. (1994)

Tumour induction	Experimental	Fibres	Results	References
	Intratracheal injection Animals: Syrian hamsters No. of animals: 20 for each treatment Dose: 2.0 mg/animal each week for 5 weeks; total 10 mg/animal Fibres tested: rock wool, fibreglass, potassium titanate fibre, calcium sulfate basic fibre, magnesium sulfate whiskers	Fibre diameter = 0.45 µm Fiber length = 22.4 µm	Tumours in hamsters that had received basic magnesium sulfate fibre Incidence: basic magnesium sulfate (9/20) > calcium sulfate fibre (3/20) > fibreglass (2/20) The primary sites of the tumours were not only in the pleural cavity but also in the intratrachial organs, kidney, adrenal gland, bladder and uterus	Adachi et al. (1991)
	Short- and long-term inhalation assay Animals: Wistar rats (male) No. of animals: 42 for each study Time of exposure: 4 weeks or 1 year (6 h/day, 5 days/week) Exposure concentration (mg/m^3): 4.0 (short-term assay) and 1.8 (long-term assay)	Two types of magnesium sulfate whiskers of different lengths (long and short) Fibre diameter (SD), µm: long = 0.44 (1.6); short = 0.31 (1.3) Fibre length (SD), µm: long = 12 (2.3); short = 12 (2.1) The whiskers had large aspect ratios, with a few non-fibrous particles	Adenomas were observed in the exposed group, but the number was not significantly greater than that of the control group Hepatocellular adenoma and carcinoma occurred somewhat more often in the long whisker group than in the control groups	Hori et al. (1994)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	In vitro study: measure of cytokines, cytoplasmic and lysosomal enzymes released from alveolar macrophages Cells: alveolar macrophages Fibres tested: crocidolite, amosite, chrysotile, anthophyllite, ceramic fibre, erionite, potassium titanate fibre, magnesium sulfate whiskers	Two types of magnesium sulfate whiskers of different lengths (long and short) Fibre diameter (SD), μm: long = 0.44 (1.6); short = 0.31 (1.3) Fibre length (SD), μm: long = 12 (2.3); short = 12 (2.1)	Magnesium sulfate whiskers induce a low increase of TNF and β-glucuronidase No significant differences were observed between long and short magnesium sulfate whiskers Activity: potassium titanate ≥ crocidolite, chrysotile, amosite > anthophyllite > erionite, ceramic fibre, magnesium sulfate whiskers	Fujino et al. (1995)

Cytotoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of LDH release from alveolar macrophages Cells: alveolar macrophages Fibres tested: crocidolite, amosite, chrysotile, anthophyllite, ceramic fibre, erionite, potassium titanate fibre, magnesium sulfate whiskers	Two types of magnesium sulfate whiskers of different lengths (long and short) Fibre diameter (SD), μm: long = 0.44 (1.6); short = 0.31 (1.3) Fibre length (SD), μm: long = 12 (2.3); short = 12(2.1)	Magnesium sulfate whiskers do not induce LDH release	Fujino et al. (1995)

Free radical generation	Experimental	Fibres	Results	References
	In vitro study (fibre-derived radicals): DNA oxidation Fibres tested: crocidolite, attapulgite, glass fibres, potassium titanate, magnesium sulfate whiskers		No increase of 8-OH-dG except for crocidolite Mannitol increases the yield of 8-OH-dG for magnesium sulfate whiskers and fibreglass	Adachi et al. (1992)

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions				

End-point	Fibre modification	Experimental conditions	Results	References
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution		Solubility study in vitro	Most soluble in vitro compared with glass fibres, ceramic fibres and potassium octatitanate whiskers	Fujino et al. (1995)
Clearance and biopersistence		Subchronic and chronic inhalation study in rats Intratracheal instillation in hamsters	Rapid clearance from the lung	Hori et al. (1994)
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis				
Genotoxicity				
Malignant transformation, alterations in growth kinetics, inhibition of differentiation				
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species		In vitro study	No increase in TNF production No significant release in LDH	Fujino et al. (1995)
Short-term animal tests				

Table 2.7 Physicochemical properties, biopersistence data and mechanistic data for polyethylene fibres

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions				
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence				
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis				
Genotoxicity				
Malignant transformation, alterations in growth kinetics, inhibition of differentiation				
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species		In vitro cytotoxicity in macrophages	Less toxic than chrysotile	Styles & Wilson (1973)
Short-term animal tests				

Table 2.8 Physicochemical properties, biopersistence data and mechanistic data for polypropylene fibres

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions				
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence		Inhalation study in rats	Number of fibres increases in the lung (segmentation)	Hesterberg et al. (1992)
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis		Inhalation study in rats	Dose-dependent minimal or mild increases in cellularity in lungs, which appear to be reversible after 90 days, especially at the lower dose	Hesterberg et al. (1992)
Genotoxicity				
Malignant transformation, alterations in growth kinetics, inhibition of differentiation				
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species		In vitro cytotoxicity in macrophages	Less toxic than chrysotile	Styles & Wilson (1973)
		Nose-only inhalation study in rats	No fibrosis	Hesterberg et al. (1992)
Short-term animal tests		Inhalation study in rats	No fibrosis	Warheit et al. (1999)

Table 2.9 Physicochemical properties, biopersistence data and mechanistic data for polyvinyl alcohol fibres

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions	PVA fibres		Range of 10–16 µm in diameter; they do not fibrillate	Harrison et al. (1999)
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence				
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis				
Genotoxicity	PVA	Reverse mutation analysis in *S. typhimurium*	No effect	Huntingdon Life Sciences (2000a)
		Reverse mutation analysis in *E. coli*	No effect	
		Reverse mutation analysis in mouse lymphoma L5178Ycells	No effect	Huntingdon Life Sciences (2000b)
		Micronucleus formation analysis in mouse bone marrow	No effect	Huntingdon Life Sciences (2000c)
Malignant transformation, alterations in growth kinetics, inhibition of differentiation				
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species				

End-point	Fibre modification	Experimental conditions	Results	References
Short-term animal tests				

Table 2.11 Physicochemical properties, biopersistence data and mechanistic data for polyvinyl chloride fibres

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions				
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence				
Fibre fragmentation in situ				
Modes of action of fibres in pulmonary/pleural carcinogenesis				
Genotoxicity	VCM	Genetic polymorphisms and sister chromatid exchanges in VCM-exposed PVC workers	Workers with susceptible metabolic and DNA repair genotypes experience an increased risk of DNA damage elicited by VCM exposure	Wong et al. (2003)
		DNA single strand breaks in PVC-exposed workers	Increased DNA single strand breaks at 5 ppm airborne VCM level	Lei et al. (2004)
Malignant transformation, alterations in growth kinetics, inhibition of differentiation	PVC-S (industrial suspension process) PVC-E (emulsion process)	Cytotoxicity study in vitro	No toxicity of PVC-S particles PVC-E particles induced significant toxicity, with EC_{50} values ranging from 0.7 to 3.7 mg/ml in the haemolysis test Most toxic particles were those that contained additives	Xu et al. (2002)
	PVC particles with their "additive-free" counterparts and with the pure additives sodium lauryl sulfate and sodium alkylbenzenesulfonate	Cytotoxicity and release of cytokines	Unwashed PVC particles induced damage of pulmonary cells Release of inflammatory mediators in vitro	Xu et al. (2003)
Cell proliferation				

End-point	Fibre modification	Experimental conditions	Results	References
Chronic inflammation, release of cytokines, growth factors, reactive species	PVC dust	Inhalation study in rats	Acute inflammation Granulomatous lesions	Agarwal et al. (1978)
Short-term animal tests	PVC dust	Inhalation study in rats, guinea-pigs and monkeys	No fibrosis No significant effects on pulmonary function Benign pneumoconiosis	Groth et al. (1981)
	PVC dust	Inhalation or intrapleural injection (20 mg/rat)	No carcinogenic effects	Wagner & Johnson (1981)
	PVC dust	Intratracheal instillation (25 mg/rat)	No histological and clinical changes of liver, kidney, spleen until 365 days post-instillation	Agarwal et al. (1991)
	Emulsion PVC particles (PVC-E3)	In vitro studies	Moderate toxicity in different pulmonary cell cultures Toxicity is probably related to the residual additives	Xu et al. (2002, 2003)
	PVC-E3 and PVC-W3 particles	Inhalation study with rats	Pulmonary toxicity of both PVC-E3 and PVC-W3 particles appeared limited	Xu et al. (2004a)
	PVC particles	Intratracheal instillation of rats	Limited potential of PVC particles in causing acute inflammatory reaction in the lung	Xu et al. (2004b)
Carcinogenicity studies	PVC dust	Intraperitoneal injection of rats	No tumours	Pott et al. (1989)
	Analysis of 12 studies of VC production and polymerization workers		Elevated risk of liver malignancies, the possibility of a 2-fold increased risk of brain and central nervous system tumours and perhaps, also, of malignancies of the lymphatic and haematopoietic system	Nicholson et al. (1984)
	Cohort study of 4806 males employed at a synthetic chemicals plant		Excess was found to be limited to adenocarcinoma and large cell undifferentiated lung cancer	Waxweiler et al. (1981)

End-point	Fibre modification	Experimental conditions	Results	References
	A total of 347 workers with occupational exposure to VCM were systemically examined using liver ultrasonography and routine liver function tests		Significantly increased risks of developing liver fibrosis	Hsiao et al. (2004)
	Epidemiological studies		Occupational exposure to PVC dust is associated with lung disorders called "PVC pneumoconiosis"	Szende et al. (1970); Arnaud et al. (1978); Antti-Poika et al. (1986); Studnicka et al. (1995)
			Occupational PVC dust exposure might affect pulmonary function and lead to a higher prevalence of small opacities on chest X-ray	Soutar & Gauld (1983); Nielsen et al. (1989); Lee et al. (1991); Ng et al. (1991)

Table 2.12 Physicochemical properties, biopersistence data and mechanistic data for potassium octatitanate fibres

	Potassium titanate whiskers	References
Fibre dimensions and aerodynamic diameter	Fibre mean diameter (SD), μm: 0.35 (1.5), range 0.5–100 Fibre geometric mean length (SD), μm: PT1 = 6.0 (2.04); range = 0.05–1	Kohyama et al. (1997); Ishihara et al. (1998)
Deposition of inhaled fibres in the different parts of the respiratory tract	Aggregated dust cells were observed in the subpleural region and around the bronchioles	Oyabu et al. (2004)
Chemical composition	$K_2Ti_8O_{17}$ No impurities were identified in the samples at a level of 1%: Fe_2O_3 was 0.2%; Co <0.1%; B 0.1%	Fujino et al. (1995); Kohyama et al. (1997); Ohyama et al. (2001)
Different kinds of potassium titanate	Potassium octatitanate, $K_2Ti_8O_{17}$ (reference sample PT1): rough surface Potassium hexatitanate, $K_2Ti_6O_{13}$ (PT2): smooth surface Similar fibre dimensions	Ishihara et al. (2002)
Surface reactivity	PT1 (rough surface) increase significantly BAL total protein, BAL cytokine-induced neutrophil attractants/growth-related gene product and fucose compared with PT2 (smooth surface) at the same dose level	Ishihara et al. (2002)

Biopersistence	Experimental	Fibres	Results	References
	Dissolution study in vitro		High persistence	Shutou et al. (1992)
	Short-term inhalation assay Animals: Wistar rats (male) Time of exposure: 4 weeks (6 h/day, 5 days/week) Exposure concentration (mg/m^3): PT1 = 1.1; TW = 1.7 Exposure concentration (fibres/ml): PT1 = 137 ± 53; TW = 73 ± 16	TW and PT1 are potassium octatitanate whiskers with the same crystalline structure, but from different factories Chemical composition: 100% $K_2O{\cdot}8TiO_2$ Fibre diameter (SD), μm: PT1 = 0.44 (1.5); TW = 0.39 (1.5) Fibre length (SD), μm: PT1 = 3.4 (2.7); TW = 5.6 (2.3)	No differences between two whiskers BHT = 3.3 months No decrease in diameter and no surface change due to dissolution were detected over the 12-month observation period Short fibres were cleared more rapidly than the longer ones (20 μm) Macrophage-mediated clearance was the main mechanism for the clearance	Yamato et al. (2002)

Biopersistence	Experimental	Fibres	Results	References
	Long-term inhalation assay Animals: male Wistar rats No. of animals: 30 Time of exposure: 1 year (6 h/day, 5 days/week) Exposure concentration (mg/m^3): 2.2 Exposure concentrations (fibres/ml): 111 ± 34	Chemical composition: 100% $K_2O{\cdot}8TiO_2$ Fibre diameter (SD), μm = 0.44 (1.5) Fibre length (SD), μm = 3.4 (2.7)	The lung burden was 2.4–0.7 mg after 1 year The clearance of PT1 after 1-year inhalation was prolonged so that the BHT was difficult to estimate	Yamato et al. (2003)
	Long-term inhalation assay Animals: male Fischer 344 rats No. of animals: 135 Time of exposure: 3, 6, 12, 18 and 24 months (6 h/day, 5 days/week) Exposure concentration: 20, 60 or 200 WHO fibres/ml		With 20 WHO fibres/ml: no accumulation in the lung With 200 WHO fibres/ml: rapid increase in lung burden with saturation of lung clearance mechanism (overloading)	Ikegami et al. (2004)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	In vitro study: measure of TNF Cells: alveolar macrophages	Fibre mean diameter (SD), μm: 0.41 (1.5) Fibre mean length (SD), μm: 2.8 (2.0)	Potassium titanate whiskers increase TNF level Potassium titanate whiskers caused the highest level of TNF production among fibres	Fujino et al. (1995)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	In vitro study: release of intracellular IL-1 Cells: RAW264.7 murine macrophage cell line Fibres tested: silicon carbide whisker, titanium oxide whisker, potassium titanate whisker, UICC crocidolite and titanium dioxide (rutile) particles	Potassium titanate whiskers from Japanese Standard Materials Fibre diameter (SD), μm: 0.35 (1.51); range 0.5–100 Fibre length (SD), μm: 6.0 (2.0); range 0.5–1	No whiskers cause a marked increase of intracellular IL-1, whereas crocidolite induced a significant increase Potassium titanate whiskers, silicon carbide whiskers, crocidolite and titanium dioxide particles induce similar production of TNF	Ishihara et al. (1998)
	Intratracheal instillation assay Animals: male Wistar rats No. of animals: 5 per treatment Fibres tested: crocidolite, RCF1, chrysotile	Fibre mean diameter (SD), μm: 0.41 (SD) Fibre mean length (SD), μm: 2.8 (2.0)	Potassium titanate whiskers produce marked pulmonary fibrosis in rats Potassium titanate whiskers produce pulmonary inflammation (increased numbers of BAL fluid neutrophils and macrophages, increased expression of TNF mRNA, peak after 3 days, and of IL-1 mRNA, peak after 1 month) The increase of gene expression is higher for potassium titanate whiskers than for crocidolite	Tsuda et al. (1997)
	Intratracheal instillation assay Animals: rats Time of exposure: 4 weeks		IL-1 and IL-6 gene expression was accelerated in alveolar macrophages and lung tissue The effect of the potassium octatitanate whiskers was similar to the effect of crocidolite Potassium octatitanate whiskers exhibit high fibrotic potential	Morimoto et al. (1999)
			Crocidolite was the most potent fibrogenic agent and was 10 times more fibrogenic than potassium octatitanate (Fybex) in terms of exposure	Lee et al. (1981)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Intratracheal instillation assay Animals: male Wistar rats No. of animals: 20 for each treatment Fibres tested: silicon carbide whisker, titanium oxide whisker, potassium titanate whisker, UICC crocidolite, and titanium dioxide (rutile) particles	Potassium titanate whiskers from Japanese Standard Materials Fibre diameter (SD), μm: 0.35 (1.51); range 0.5–100 Fibre length (SD), μm: 6.0 (2.0); range 0.5–1	At 2 months, all whiskers cause very slight fibrous reactions with inflammatory cell aggregation around the resident fibres	Ishihara et al. (1998)
	Intratracheal instillation assay Animals: male Wistar rats No. of animals: 4 for each treatment Doses: 0.2 and 1.0 mg	Potassium octatitanate $K_2Ti_8O_{17}$ (PT1, from Japanese Standard Materials) and potassium hexatitanate, $K_2Ti_6O_{13}$ (PT2) PT1 has an irregular and rough surface, whereas PT2 has a smooth surface Geometric mean length (μm): PT1: 6.0; PT2: 5.0 Geometric mean width (μm): PT1: 0.35; PT2: 0.31	Total protein concentration in BAL increased significantly from day 1 BAL total protein and fucose in group PT1 increased significantly compared with those in group PT2 at a dose level of 1.0 mg A dose-independent increase of gamma-glucuronidase activity and decrease of SOD activity were observed in both fibres BAL tumour necrosis factor-f (TNF-f) increased significantly in animals treated with 1.0 mg dosage of PT1 and PT2 on day 1 On day 1, BAL cytokine-induced neutrophil attractants/growth-related gene product increased significantly in the PT1 group treated with 0.2 and 1.0 mg dosages	Ishihara et al. (2002)
	Long-term inhalation assay Animals: male Wistar rats No. of animals: 30 Time of exposure: 1 year (6 h/day, 5 days/week) Exposure concentration (mg/m^3): 2.2 Exposure concentration (fibres/ml): 111 ± 34		Increase of IL-6 and TNF gene expression in lung tissue Mild fibrotic change around fibre-laden macrophages in the rat lungs	Tsuda et al. (1998)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Long-term inhalation assay Animals: Wistar rats No. of animals: 35 for each treatment Time of exposure: 13 weeks (6 h/day, 5 days/week) Exposure concentration (mg/m^3): 0.1, 10, 100 Exposure concentration (fibres/ml): 1700 (123 WHO), 5900 (952 WHO), 112 000 (7440 WHO)	Fibre mean diameter (SD), µm: 0.48 (1.5) Fibre mean length (SD), µm: 2.8 (2.0)	No effect for 0.1 mg Slight fibrotic thickening in the alveolar ducts and adjoining alveoli, with proliferating fibroblasts and microgranulomas dose dependent (100 >> 10 mg/m^3)	Ikegami et al. (????)

Cytoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of cytokines, cytoplasmic and lysosomal enzymes released from alveolar macrophages Cells: alveolar macrophages Fibres tested: crocidolite, amosite, chrysotile, anthophyllite, ceramic fibre, erionite, potassium titanate fibre, magnesium sulfate whiskers	Fibre length = 2.8 µm	Potassium titanate induces LDH release Activity: potassium titanate = crocidolite, chrysotile, amosite > anthophyllite > erionite, ceramic fibre, magnesium sulfate whiskers (no activity)	Fujino et al. (1995)
	In vitro study: measure of LDH release and cell viability Cells: RAW264.7 murine macrophage cell line Fibres tested: silicon carbide whisker, titanium oxide whisker, potassium titanate whisker, UICC crocidolite and titanium dioxide (rutile) particles	Fibre diameter (SD), µm: 0.35 (1.51); range 0.5–100 Fibre length (SD), µm: 6.0 (2.0); range 0.5–1	The three types of whiskers caused an increase in LDH transudation and a decrease of cell viability at 24 h of incubation in a dose-dependent manner There was no significant difference found among the three kinds of whiskers Activity < crocidolite	Ishihara et al. (1998)

Tumour induction	Experimental	Fibres	Results	References
	Intraperitoneal injection Animals: hamsters		High incidence of mesothelioma	Lee et al. (1981)
	Intraperitoneal injection assay Animals: F344 rats No. of animals: 13 for each treatment Doses: 5 and 10 mg Fibres tested: chrysotile, glass wool, rock wool, refractory fibre, silicon carbide whisker, titanium oxide (rutile) whisker, wollastonite		Incidence of peritoneal mesothelioma at the end of the experiment was 85% for 10 mg UICC chrysotile B, 77% for 10 mg of potassium titanate whisker, 70% for 5 mg of silicon carbide whisker, 20% for 5 mg of potassium titanate whisker, 20% for 20 mg of refractory fibre 2 and 10% for 20 mg of refractory fibre 1	Adachi et al. (2001)
	Long-term inhalation assay Animals: male Fischer 344 rats No. of animals: 135 Time of exposure: 3, 6, 12, 18 and 24 months (6 h/day, 5 days/week)		Neither exposure-related pulmonary neoplasm nor mesothelioma was observed in 24 months of exposure	Ikegami et al. (2004)

Free radical release and genotoxicity	Experimental	Fibre	Results	References
	In vitro study (fibre-derived radicals): DNA oxidation Fibres tested: crocidolite, glass fibres, potassium titanate		No formation of 8-OH-dG except for crocidolite	Nejjari et al. (1993)

Free radical release and genotoxicity	Experimental	Fibre	Results	References
	In vitro study (cell-derived radicals): DNA oxidation Cells: J774 cell line (murine reticulum cell sarcoma) Fibres tested: crocidolite, amosite, chrysotile ceramic fibres, glass fibres Concentration: 100 µg/ml		No increase of 8-OH-dG except for crocidolite and amosite	Murata-Kamiya et al. (1997)
	In vitro study: DNA oxidation Fibres tested: crocidolite, attapulgite, glass fibres		No increase of 8-OH-dG except for crocidolite + $FeSO_4$ increases the yield of 8-OH-dG by attapulgite, fibreglass, potassium titanate more than by asbestos	Adachi et al. (1992)
	In vitro study (cell-derived radicals): release of superoxide Cells: human monocyte-derived macrophages Fibres tested: glass wool, rock wool, micro glass fibre, refractory ceramic fibre 1.1, 8.7, refractory mullite fibre, potassium titanium whisker, silicon carbide whisker, titanium oxide whisker, wollastonite Concentration: 10 mg/ml	Potassium titanate whiskers from Japanese Standard Materials Fibre diameter (SD), µm: 0.35 (1.51); range 0.5–100 Fibre length (SD), µm: 6.0 (2.0); range 0.5–1	All fibres (except wollastonite) induced release of superoxide Each response was almost completely inhibited by SOD The release of superoxide occurs nonspecifically for various types of mineral fibres depending on fibre length	Ohyama et al. (2001)

Table 2.13 Physicochemical properties, biopersistence data and mechanistic data for synthetic vitreous fibres

1a: Fibrous glass (all papers)

	MMVF10	MMVF10	MMVF10	MMVF10a	MMVF10a	B-01/09 (GF)	CM44 (similar to "C" fibres)
References	Xie et al. (2000)	Brown et al. (2000)	Droger et al. (2000)	Bermudez et al. (2003)	Bermudez et al. (2003)	Creutzenberg et al. (1997)	Bottin et al. (2003)
Mean length, µm		85.24% >10 67.17% >20	Median: 16.3 (1.8–74.0)	Geometric mean length (GML) = 12.5 ± 2.5	GML = 12.5 ± 2.5	50% >8.6[5] 902 WHO fibres/ml[5] 1324 particles/ml[5]	GML = 3.8 ± 1.97
Mean diameter, µm				Geometric mean diameter (GMD) = 0.93 ± 1.5	GMD = 0.93 ± 1.5	50% <0.99	GMD = 0.34 ± 1.53
Cell type/animals	Rat tracheal explant	In vitro	Rat, Syrian hamster alveolar macrophages	F344 rat [4]	Hamster, Syrian golden[5]	Female Wistar rats [Crl(WI)BR][6]	Transgenic Big Blue rats, male *LacI* F344 rats exposed to aerosols[9]
Clearance/ biopersistence							
Mucociliary mvt							
Transport	Yes, but with higher concentrations of TNF-α[1]			GML = 6.9 µm GMD = 0.71 µm Total no. = 50.1 × 10^6 (7.6 × 10^3/cm^2) down to 56% Length ratio >5/<5 µm =1/2; constant with time	GML = 6.9 µm GMD = 0.68 µm Total no. = 6.4 × 10^6 (2.3 × 10^3/cm^2) down to 10% Length ratio >5/<5 µm =1/2; goes to shorter and thinner with time	1.61 × 10^6 WHO fibres[7] $37^{(a)}$/$57^{(b)}$	50% fibres >20 µm cleared in 12.8 days Lung burden 90% <12.8 µm (1 day); <7.9 µm (90 days)

	MMVF10	MMVF10	MMVF10	MMVF10a	MMVF10a	B-01/09 (GF)	CM44 (similar to "C" fibres)
Pleural translocation				GML = 8.3 ± 1.6 µm GMD = 0.63 ± 1.5 µm Total no. = 1.2×10^3	GML = 10.3 ± 1.9 µm GMD = 0.67 ± 1.5 µm Total no. = 1.4×10^3		
Dissolution in situ							
Fibre fragmentation in situ							
Mode of action							
Phagocytosis			Yes, 10.4 µm (rat); 8.8 µm (hamster)[3] Fibre-free alveolar macrophages: 14% (rat); 22% (hamster)[3]				Yes, found in alveolar macrophages
Point mutations							No mutation in *lacI* and *cII* genes
Base, DNA damage							
Deletions							
Effects on mitosis							
Chromosome damage							
Gene transfer							
Malignant transformation							
Growth alteration							
Altered differentiation							

	MMVF10	MMVF10	MMVF10	MMVF10a	MMVF10a	B-01/09 (GF)	CM44 (similar to "C" fibres)
Activation of GFs							
Activation of GFR							
Signalling pathways							
Death: apoptosis, necrosis			Low ↑ LDH release (rat) No ↑ LDH release (hamster)				
Cell influx in BAL/BAL parameters				BAL = macrophages, neutrophils, lymphocytes Pleural lavage (PL) = macrophages; few neutrophils, lymphocytes	Macrophage (≈ rats); neutrophils, lymphocytes lower than in rats PL = similar to rats	No increase[8]	
Cytokine release				Enzyme release No release	No release No release		
Release of growth factors							
ROS production		Yes, greater than with RCF and amosite[2]					
Short-term animal tests				Yes (diaphragm, at 4 weeks)	Yes (costal wall, at 4 weeks)		
Carcinogenicity studies							

[1] Adhesion to tracheal explant. Role of TNF-α studied by the addition of increased concentrations of TNF-α.
[2] Depletion of antioxidants, GSH and ascorbate in lung lining fluid material (from rats) and in pure solutions of these molecules. Equivalent number of fibres. Authors conclude that antioxidant depletion in these conditions is not an indicator of pathological potential.
[3] Videomicroscopy. Median size of completely phagocytized fibres.

[4] 0, 4 and 12 weeks of exposure and 12 weeks postexposure. Aerosol: 4.4 ± 1.6 mg/m^3; 761 fibres/cm^3; 13% non-fibrous (number).
[5] Values for 30 mg/m^3.
[6] Inhalation study, nose-only. Exposure time: 1 week (30 mg/m^3) or 3 weeks (40 mg/m^3), except RCF1: 3 weeks (40 mg/m^3).
[7] Retention per lung at day 93 after the end of exposure. Clearance half-time: [(a)] calculated from fibre retention measurements of days 3–365; [(b)] from measurements with tracer particles ($^{46}Sc_2O_3$); control: half-time = 66 days.
[8] Cells: PMN; other parameters: LDH, proteins, gamma-glutamyl transferase.
[9] 6.3 ± 2.7 mg/m^3 (601 ± 338 WHO fibres/cm^3 (PCOM); 72 ± 54 fibres/cm^3 >20 µm of length).

1b: Fibrous glass, continued (all papers)

Fibre type	Code 100/475	104/475[2]	104E	GW Code A	Glass wool	MN#100 (glass fibre)	Glass wool
References	Brown et al. (2000)	Cullen et al. (2000)	Cullen et al. (2000)	Zoller & Zeller (2000)	Cavallo et al. (2004)	Fukada et al. (1987)	Nishiike et al. (2005)
Mean length, µm	50% >10 19.32% >20	2911 fibres/ml Length <5 3977 total fibres/ml (58 fibres 15–20 µm in length)	1381 fibres/ml length <5 2354 total fibres/ml (76 fibres 15–20 µm in length)	86.5% >5	(arithmetic) 57.3		20.0 ± 2.58
Mean diameter, µm				84% <1	(arithmetic) 4.3		0.88 ± 3.10
Cell type/animals	In vitro[1]	Wistar rats	Wistar rats	HL-60-M[7]	MeT-5A	Rats[11]	RAW264.7 and J774
Clearance/ biopersistence							
Mucociliary mvt							
Transport		14 982 × 10^6 fibres (95 fibres 15–20 µm in length) (10^6 fibres >15 µm in length)[5] 4244 fibres (58 fibres >15 µm in length)[6]	Clearance half-time = 7.1 months 11 028 × 10^6 fibres (167 fibres 15–20 µm in length; 250 fibres >15 µm in length)[5] 3271 fibres (35 fibres >15 µm in length)[6]				

Fibre type	Code 100/475	104/475[2]	104E	GW Code A	Glass wool	MN#100 (glass fibre)	Glass wool
Pleural translocation							
Dissolution in situ		Substantial chemical changes after 12 months' recovery	No chemical changes after 12 months' recovery				
Fibre fragmentation in situ							
Mode of action							
Phagocytosis					Yes (SEM)		
Point mutations							
Base, DNA damage					Slight direct and oxidative; not significant[10]		
Deletions							
Effects on mitosis							
Chromosome damage							
Gene transfer							
Malignant transformation							
Growth alteration							
Altered differentiation							
Activation of GFs							
Activation of GFR							
Signalling pathways							
Death: apoptosis, necrosis							

Fibre type	Code 100/475	104/475[2]	104E	GW Code A	Glass wool	MN#100 (glass fibre)	Glass wool
Cell influx in BAL/BAL parameters							
Cytokine release							
Release of growth factors							
ROS production	Yes, greater than with RCF and amosite			Yes; ↓ after preincubation[8,9]			NO_2^- production (RAW264.7 and J774)[12] ↓ GSH; ↑GSSG[12] ↑ RSNO (RAW264.7 and J774)[12] No $O_2^{\bullet}$ generation[12]
Short-term animal tests							
Carcinogenicity studies		Fibrosis much lower than with 104E3 10.5% (4/38) lung tumours[3] 0% mesotheliomas[3] 8/24 mesotheliomas[4]	Alveolar fibrosis; bronchoalveolar hyperplasia[3] 23.2% (7/43) lung tumours[3] 4.7% mesotheliomas[3] 21/24 mesotheliomas[4]			Fibrosis = 8.7% (2/23) Hyperplasia = 8.7% (2/23) Mesothelioma = 21.7% (5/23)	

[1] Depletion of antioxidants, GSH and ascorbate in lung lining fluid material (from rats) and in pure solutions of these molecules. Equivalent number of fibres.
[2] Inclusion of published data (Davis et al., 1996).
[3] Inhalation chamber; 1000 fibres/ml >5 µm in length for 12 months; recovery period = 12 months. Lung tumours in controls = 5.3% (2/38); mesotheliomas 0%.
[4] Intraperitoneal injection studies (10^9 fibres). No mesothelioma in controls.
[5] Total fibres deposited per lung after 12 months' exposure.
[6] Total fibres deposited per lung after 12 months' exposure + 12 months' recovery.
[7] Differentiated promyelocytic cell line (1,25-dihydroxyvitamin D3).

[8] In vivo cells in culture; chemoluminescence. Positive control = quartz (2.2 m^2/g). Assay with and without preincubation in unbuffered physiological saline for 4 weeks.
[9] When decreased after preincubation: basic pH of solutes When enhanced after preincubation: acid pH of solutes.
[10] Comment: From the figures, direct breakage enhancement with all particles.
[11] Intrapleural inoculation of 20 mg. Data 25 months' postexposure.
[12] NO_2^- release (Greiss reagent; spectrophotometry) in both cell types; GSH and GSSG measurement in RAW264.7; measurement of S-nitrosothiols (RSNO); measurement of $O_2^{\bullet}$: cytochrome C reduction, with and without SOD in RAW264.7.

2a: Mineral wool (all papers)

Fibre type	MMVF21	MMVF21	D6 (equivalent to MMVF21)	CMS[8]	CMS[8]	CMZS[8]	CMZS[8]	Stone wool A (Saint Gobain)	Stone wool B1 (Saint Gobain)	Stone wool B2 (Saint Gobain)	Stone wool C (Saint Gobain)
References	Creutzenberg et al. (1997)	Droger et al. (2000)	Kamstrup et al. (2002)	Bellmann et al. (2002)	Brown et al. (2002)	Bellmann et al. (2002)	Brown et al. (2002)	Dika Ngua et al. (2005)	Dika Ngua et al. (2005)	Dika Ngua et al. (2005)	Dika Ngua et al. (2005)
Mean length, µm	50% >7.6[1] 879 WHO fibres/ml[1] 1423 particles/ml[1]		GML = 8.5; median = 7.8 WHO >20 µm in length = 18%	644 WHO fibres/ml 152 fibres/ml >20 µm in length Particles = 70.7 mg/m^3		535 WHO fibres/ml 152 fibres/ml >20 µm in length Particles = 49.8 mg/m^3		GML = 14.54	GML = 11.30	GML = 11.10	GML = 11.66
Mean diameter, µm	50% <0.95		GMD = 0.8; median = 0.87					GMD = 0.85	GMD = 0.62	GMD = 0.88	GMD = 0.92
Cell type/ animals	Female Wistar rats [Crl:(WI)BR][2]	Rat, Syrian hamster alveolar macrophages	Female Wistar rats[1]	Male Fischer 344 rats	Rats	Male Fischer 344 rats	Rats	U937[13]	U937[13]	U937[13]	U937[13]
Clearance/ biopersistence											
Mucociliary mvt											

Fibre type	MMVF21	MMVF21	D6 (equivalent to MMVF21)	CMS[8]	CMS[8]	CMZS[8]	CMZS[8]	Stone wool A (Saint Gobain)	Stone wool B1 (Saint Gobain)	Stone wool B2 (Saint Gobain)	Stone wool C (Saint Gobain)
Transport	8.82×10^6 WHO fibres[3] 92[(a)]/102[(b)]		Half-time = 6 days Traditional MMVF21 = 65 days (WHO fibres); 92 days (long fibres)[5]	WHO: L = 9.2; LN = 0.063[9] >20 µm in length: L = 0.01; LN = 0[9] P = 0.543 mg/lung[9] No ↑[10] WHO ~50[11] >20 µm in length ~90[11]		WHO: L = 5.0; LN = 0.016[9] >20 µm in length: L = 0.23; LN = 0.01[9] P = 0.181 mg/lung[9] No ↑[10] WHO ~10[11] >20 µm in length ~50[11]					
Pleural translocation											
Dissolution in situ											
Fibre fragmentation in situ											
Mode of action											
Phagocytosis		Yes, 7.7 µm (both rat and hamster)[5] Fibre-free alveolar macrophages: 8% (rat); 12% (hamster)						Yes (SEM)	Yes (SEM)	Yes (SEM)	Yes (SEM)

Fibre type	MMVF21	MMVF21	D6 (equivalent to MMVF21)	CMS[8]	CMS[8]	CMZS[8]	CMZS[8]	Stone wool A (Saint Gobain)	Stone wool B1 (Saint Gobain)	Stone wool B2 (Saint Gobain)	Stone wool C (Saint Gobain)
Point mutations											
Base, DNA damage											
Deletions											
Effects on mitosis											
Chromosome damage											
Gene transfer											
Malignant transformation											
Growth alteration					No		No				
Altered differentiation											
Activation of GFs											
Activation of GFR											
Signalling pathways											
Death: apoptosis, necrosis		↑ LDH release (rat) No ↑ LDH release (hamster)						Significantly enhanced[14]	Enhanced[14]	Enhanced[14]	Enhanced[14]
Cell influx in BAL/BAL parameters	Increase[4]				No increase[12]		No increase[12]				
Cytokine release											

Fibre type	MMVF21	MMVF21	D6 (equivalent to MMVF21)	CMS[8]	CMS[8]	CMZS[8]	CMZS[8]	Stone wool A (Saint Gobain)	Stone wool B1 (Saint Gobain)	Stone wool B2 (Saint Gobain)	Stone wool C (Saint Gobain)
Release of growth factors											
ROS production											
Short-term animal tests											
Carcinogenicity studies			Mean survival = 474 days (controls = 536 days)[7] 56% mesotheli-omas (32/57) (none in controls)[7]								

[1] Values for 30 mg/m^3.
[2] Inhalation study, nose-only. Exposure time: 1 week (30 mg/m^3) or 3 weeks (40 mg/m^3), except RCF1: 3 weeks (40 mg/m^3).
[3] Retention per lung at day 93 after the end of exposure. Clearance half-time: [(a)] calculated from fibre retention measurements of days 3–365; [(b)] from measurements with tracer particles ($^{46}Sc_2O_3$); control: half-time = 66 days.
[4] Cells: PMN; other parameters: LDH, proteins, gamma-glutamyl transferase.
[5] Videomicroscopy. Median size of phagocytized fibres.
[6] Inhalation/biopersistence assay (Kamstrup et al., 1998).
[7] A single intraperitoneal injection of 0.5×10^9 WHO fibres. Control = saline. Delay = until survival (about 50 rats per group).
[8] High-temperature insulation wool. CMS = calcium magnesium silicate; CMZS = calcium magnesium zirconium silicate.
[9] Inhalation: 6 h/day, 5 days/week, for 90 days. Analysis of fibres in the lung (L) and in lymph nodes (LN); and mass of particles (P) retained in the lung from 0.1 to 12 months (quoted in this table at 6 months),
[10] Clearance half-time of labelled particles ($^{46}Sc_2O_3$) after 5 days and 6 months.
[11] Clearance half-time of WHO fibres and fibres >20 µm in length.
[12] Cell influx and total proteins in BAL.
[13] Monocytic cell line (histiocytic lymphoma) differentiated with 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA).
[14] Enzyme-linked immunosorbent assay (ELISA) for apoptosis (Roche).

2b: Mineral wool, continued (all papers)

Fibre type	HT (Roxul®) (MMVF34)	Stone wool code G	Stone wool HT-N	Rock wool	Danish rock wool	Rock wool	Japan RW1
References	Kamstrup et al. (2002)	Zoller & Zeller (2000)	Zoller & Zeller (2000)	Cavallo et al. (2004)	Cavallo et al. (2004)	Kudo et al. (2003)	Kim et al. (2001)
Mean length, µm	GML = 10.7; median = 9.9 WHO fibres >20 µm in length = 28%			(arithmetic) 52.1	96.9	16.5 ± 2.51	16.5 ± 2.5 (1.7×10^3 fibres/µg)
Mean diameter, µm	GMD = 0.65; median = 0.67			(arithmetic) 2.3	3.7	1.8 ± 2.3	
Cell type/animals	Female Wistar rats	HL-60-M[3]	HL-60-M[3]	MeT-5A	MeT-5A	Fischer rat alveolar macrophages	Rat alveolar macrophages
Clearance/ biopersistence							
Mucociliary mvt							
Transport	Half-time: HT fibres: 25 days (WHO fibres) and 6 days (fibres >20 µm in length)[1]						
Pleural translocation							
Dissolution in situ							
Fibre fragmentation in situ							
Mode of action							
Phagocytosis				Yes (SEM)	Yes (SEM)	Yes (SEM, TEM)	
Point mutations							

Fibre type	HT (Roxul®) (MMVF34)	Stone wool code G	Stone wool HT-N	Rock wool	Danish rock wool	Rock wool	Japan RW1
Base, DNA damage				Slight direct[6] Significant oxidative	Slight direct and oxidative; not significant[6]		
Deletions							
Effects on mitosis							
Chromosome damage							
Gene transfer							
Malignant transformation							
Growth alteration							
Altered differentiation							
Activation of GFs							
Activation of GFR							
Signalling pathways							
Death: apoptosis, necrosis						Cytotoxicity: no modification of relaxation, no LDH release Apoptosis: No DNA fragmentation	Cytotoxicity[7]: yes, smaller than RF2 ATP reduction greater than RF2 Reduction of oxygen consumption by mitochondria
Cell influx in BAL/BAL parameters							
Cytokine release							
Release of growth factors							

Fibre type	HT (Roxul®) (MMVF34)	Stone wool code G	Stone wool HT-N	Rock wool	Danish rock wool	Rock wool	Japan RW1
ROS production		Yes; ↓ after preincubation[4,5]	Yes; ↑ after preincubation[4,5]				
Short-term animal tests							
Carcinogenicity studies	Mean survival = 646 days (controls = 672 days)[2] 0% mesotheliomas (0/50) (none in controls)						

[1] Inhalation biopersistence assay (Kamstrup et al., 1998).
[2] A single intraperitoneal injection of 0.5×10^9 WHO fibres. Control = saline. Delay = until survival (about 50 rats per group).
[3] Differentiated promyelocytic cell line (1,25-dihydroxyvitamin D3).
[4] In vivo cells in culture; chemoluminescence. Positive control = quartz (2.2 m^2/g). Assay with and without preincubation in unbuffered physiological saline for 4 weeks.
[5] When decreased after preincubation: basic pH of solutes When enhanced after preincubation: acid pH of solutes.
[6] Comment: From the figures, direct breakage enhancement with all particles.
[7] MTT assay; LDH release; ATP level; mitochondrial activity (oxygen consumption).

3a: Ceramic fibres (all papers)

Fibre type	RCF1	RCF1[4]	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1a	RCF3[4]	RCF (equivalent to RCF3)	RCF (equivalent to RCF3)
Reference	Creutzenberg et al. (1997)	Elias et al. (2002)	Creutzenberg et al. (1997)	Brown et al. (2000)	Bellmann et al. (2001)	Mast et al. (2000)[1] [1]	Mast et al. (2000)[11]	Mast et al. (2000)[11]	Mast et al. (2000)[11]	Bellmann et al. (2001)	Elias et al. (2002)	Brown et al. (2002)	Bellmann et al. (2002)

Fibre type	RCF1	RCF1[4]	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1a	RCF3[4]	RCF (equivalent to RCF3)	RCF (equivalent to RCF3)
Mean length, µm	50% >6.3 µm 679 WHO fibres/ml 1298 particles/ml	GML: 7.32 ± 3.01 (14.3×10^6 fibres/mg; 40.7% >10 µm in length)	50% >6.3 679 WHO fibres/ml 1298 particles/ml	77.36% >10 45.27% >20	130 ± 24 fibres/ml >20 µm in length 682 ± 171 fibres/ml >5 µm in length 552 ± 147 WHO fibres/ml Particles: 51.2 ± 8.1 mg/m^3	GML: 13.5 ± 2.6 Total fibres: 36/ml; WHO fibres: 26 ± 12/ml	GML: 13.9 ± 2.5 Total fibres: 91/ml; WHO fibres: 75 ± 35/ml	GML: 13.8 ± 2.5 Total fibres: 162/ml; WHO fibres: 120 ± 135/ml	GML: 135.9 ± 2.4 Total fibres: 234/ml; WHO fibres: 187 ± 53/ml	125 ± 48 fibres/ml >20 µm in length 488 fibres/ml >5 µm in length 363 ± 122 WHO fibres/ml Particles: 25.8 ± 5.8 mg/m^3	GML: 8.52 ± 3.39 (9.55×10^6 fibres/mg; 44.7% >10 µm in length)		515 WHO fibres/ml 137 fibres/ml >20 µm in length Particles: 58.6 mg/m^3
Mean diameter, µm	50% <0.82	GMD: 0.60 ± 2.26	50% <0.82			0.80 ± 2.06	0.80 ± 2.03	0.80 ± 1.99	0.82 ± 1.89		GMD: 0.54 ± 2.34		
Cell type/animals	Female Wistar rats [Crl:(WI)BR][1]	SHE	Female Wistar rats [Crl:(WI)BR][1]	In vitro	Female Wistar rats[8]	Rats	Rats	Rats	Rats	Female Wistar rats[8]	SHE	Rats	Male Fischer 344 rats[15]
Clearance/ biopersistence													
Mucociliary mvt													

Fibre type	RCF1	RCF1[4]	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1a	RCF3[4]	RCF (equivalent to RCF3)	RCF (equivalent to RCF3)
Transport	13.08×10^6 WHO fibres[2] 113[(a)]:1200[(b)]		13.08×10^6 WHO fibres[2] 113[(a)]:1200[(b)]		Half-time = 1200 days (control = 66 days)[9] Charge of lung-associated lymph nodes = 0.306×10^6 WHO fibres 12 months postexposure					Half-time = 60 days (control = 60 days)[9] Charge of lung-associated lymph nodes in WHO fibres = 0.017×10^6/rat, 12 months post-exposure)			WHO fibres: L = 22.8; LN = 0.612[16] >20 µm in length: L = 3.15; LN = 0.013[16] P = 2.199 mg/lung[16] High ↑[17] WHO ~320[18] >20 µm length ~150[18]
Pleural translocation													
Dissolution in situ													
Fibre fragmentation in situ													
Mode of action													
Phagocytosis													
Point mutations													
Base, DNA damage													
Deletions													
Effects on mitosis													
Chromosome damage													
Gene transfer													

Fibre type	RCF1	RCF1[4]	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1a	RCF3[4]	RCF (equivalent to RCF3)	RCF (equivalent to RCF3)
Malignant transformation		Yes, attenuated by iron coating									Yes, the most active, attenuated by iron coating		
Growth alteration												Yes[14]	
Altered differentiation													
Activation of GFs													
Activation of GFR													
Signalling pathways													
Death: apoptosis, necrosis		Cytotoxicity: yes			In BAL: ↑ LDH					In BAL: ↑ LDH	Cytotoxicity: yes, the most active		
Cell influx in BAL/BAL parameters	Increase[3]		Increase[3]		PMNs influx; 18.9% to 3.4% between 3 days and 12 months post-exposure					PMNs influx; 15.2% to 1.8% between 3 days and 12 months post-exposure		Slight increase	
Cytokine release													
Release of growth factors													

Fibre type	RCF1	RCF1[4]	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1	RCF1a	RCF3[4]	RCF (equivalent to RCF3)	RCF (equivalent to RCF3)
ROS production		No without H_2O_2; faint spectrum with H_2O_2[5] Yes, enhanced when iron-coated[6]		Yes[7]	In BAL: ↑ GSH and ↑ GSSG; ↑ in gamma-glutamyl transferase					In BAL: ↑ GSH and ↓ GSSG; ↑ in gamma-glutamyl transferase	No without H_2O_2; faint spectrum with H_2O_2[5] Yes, enhanced when iron-coated[6]		
Short-term animal tests					Very slight fibrosis, up to 12 months More inflammatory cells[10]					Very slight fibrosis, up to 12 months (duration of analysis)[10]			
Carcinogenicity studies						1.6%[12] 8.1%[13]	3.9%[12] 12.6%[13]	1.6%[12] 10.2%[13]	13.0%[12] 13.8%[13]				

[1] Inhalation study, nose-only. Exposure time: 1 week (30 mg/m^3) or 3 weeks (40 mg/m^3), except RCF1: 3 weeks (40 mg/m^3).
[2] Retention per lung at day 93 after the end of exposure. Clearance half-time: [(a)] calculated from fibre retention measurements of days 3–365; [(b)] from measurements with tracer particles ($^{46}Sc_2O_3$); control: half-time = 66 days.
[3] Cells: PMN; other parameters: LDH, proteins, gamma-glutamyl transferase.
[4] Either untreated or iron-coated.
[5] In vitro: free radical release (5,5-dimethyl-1-pyrroline *N*-oxide–hydroxyl radical, DMPO-OH); EPR in the presence of formate.
[6] In vitro: catalytic decomposition of H_2O_2 (measurement of H_2O_2 consumed).
[7] Depletion of antioxidants, GSH and ascorbate in lung lining fluid material (from rats) and in pure solutions of these molecules. Equivalent number of fibres. Authors conclude that antioxidant depletion in these conditions is not an indicator of pathological potential.
[8] Inhalation, 6 h/day, 5 days/week, 3 weeks. Postexposure, up to 12 months.
[9] Macrophage-mediated clearance of labelled particles. Two different studies.
[10] Histological analysis (two animals).
[11] Review of chronic inhalation studies in rats; RCC studies.
[12] Bronchoalveolar hyperplasia: 1.2% in controls.
[13] Lung tumours: adenomas + carcinomas: 0.8% in controls.
[14] Proliferation in the terminal airways.
[15] Inhalation: 6 h/day, 5 days/week, for 90 days.
[16] Analysis of fibres in the lung (L) and in lymph nodes (LN); and mass of particles (P) retained in the lung from 0.1 to 12 months (quoted in this table at 6 months).

[17] Clearance half-time of labelled particles ($^{46}Sc_2O_3$) after 5 days and 6 months.
[18] Clearance half-time of WHO fibres and fibres >20 µm in length.

3b: Ceramic fibres, continued (all papers)

Fibre type	RCF4	RCF4	RCF4[2]	RF1 (Japan)	RF1	RCF	Ceramic	RCF	Japan RF2
Reference	Brown et al. (2000)	Brown et al. (2000)	Elias et al. (2002)	Motimoto et al. (2001)	Nishiike et al. (2005)	Cavallo et al. (2004)	Morimoto et al. (1994)	Dopp et al. (1997)	Kim et al. (2001)
Mean length, µm	59.35% >10 17.96% >20	59.35% >10 17.96% >20	GML: 5.54 ± 2.11 (15.55×10^6 fibres/mg; 21.5% >10 µm in length)	GML: 12.0 ± 2.36 (29% >20 µm in length)	12.0 ± 2.36	44.5		(average) 12.03	11.0 ± 1.96 (8.7×10^3 fibres/µg)
Mean diameter, µm			GMD: 0.88 ± 1.78	GMD: 0.77 ± 2.53 (51% <1 µm in diameter)	0.77 ± 2.53	3.3	Mass median aerodynamic = 4.4 ± 2.0	(average) 0.90	
Cell type/animals	In vitro	In vitro	SHE	Male Wistar rats[5]	RAW264.7 and J774[6]	MeT-5A	Male Wistar rats	Human amniotic fluid cells	Rat alveolar macrophages
Clearance/ biopersistence									
Mucociliary mvt									
Transport							Increased phagocytic activity of alveolar macrophages towards heat-inactivated yeasts		
Pleural translocation									

Fibre type	RCF4	RCF4	RCF4[2]	RF1 (Japan)	RF1	RCF	Ceramic	RCF	Japan RF2
Dissolution in situ									
Fibre fragmentation in situ									
Mode of action									
Phagocytosis						Yes (SEM)			
Point mutations									
Base, DNA damage						Direct significant (only at low dose; decrease at highest doses), no oxidative[7]			
Deletions									
Effects on mitosis									
Chromosome damage								Breakage and hyperploidy[8]	
Gene transfer									
Malignant transformation			Weakly active						
Growth alteration									
Altered differentiation									
Activation of GFs									

Fibre type	RCF4	RCF4	RCF4[2]	RF1 (Japan)	RF1	RCF	Ceramic	RCF	Japan RF2
Activation of GFR									
Signalling pathways									
Death: apoptosis, necrosis			Cytotoxicity: yes						Cytotoxicity[9]: yes; greater than RW1 ATP reduction smaller than RW1 Reduction of oxygen consumption by mitochondria
Cell influx in BAL/BAL parameters									
Cytokine release				No release of mRNA of TNF-α, IL-6; enhancement of TGF-β No histological changes					
Release of growth factors									

Fibre type	RCF4	RCF4	RCF4[2]	RF1 (Japan)	RF1	RCF	Ceramic	RCF	Japan RF2
ROS production	Yes[1]	Yes[1]	No without H_2O_2; faint spectrum with H_2O_2[3] Yes, enhanced when iron-coated[4]		NO_2^- production (RAW264.7 and J774) ↑ GSH; ↑GSSG (not significant) ↑ RSNO (RAW264.7 and J774) No $O_2^{\bullet}$ generation				
Short-term animal tests									
Carcinogenicity studies									

[1] Depletion of antioxidants, GSH and ascorbate in lung lining fluid material (from rats) and in pure solutions of these molecules. Equivalent number of fibres. Authors conclude that antioxidant depletion in these conditions is not an indicator of pathological potential.
[2] Either untreated or iron-coated.
[3] In vitro: free radical release (DMPO-OH); EPR in the presence of formate.
[4] In vitro: catalytic decomposition of H_2O_2 (measurement of H_2O_2 consumed).
[5] Intratracheal: 2 mg. Measurements 4 weeks after instillation. Inhalation: 6 h/day, 5 days/week, 1 year (2.8 ± 1.0 mg/m^3; 12.3 ± 2.6 fibres/ml). Measurement of expression in the lung after 1 year inhalation.
[6] NO_2^- release (Greiss reagent; spectrophotometry) in both cell types; GSH and GSSG measurement in RAW264.7; measurement of S-nitrosothiols; measurement of $O_2^{\bullet}$: cytochrome C reduction, with and without SOD in RAW264.7.
[7] Comet assay in alkaline conditions.
[8] Micronucleus test with CREST serum. FISH chromosomes 1 and 9.
[9] MTT assay; LDH release; ATP level; mitochondrial activity (oxygen consumption).

Table 2.15 Mechanistic data, physicochemical properties and biopersistence data for wollastonite

	Wollastonite	References
Fibre dimensions	Mean fibre length = 6.0 µm Mean fibre diameter = 0.7 µm	Fujino et al. (1995)
Chemical composition	$CaSiO_3$ Wollastonite is an acicular mineral that occurs in triclinic and monoclinic varieties Chemical analysis: SiO_2 49–51%; CaO 44–46%; MgO 0.3–1%; Al_2O_3 0.3–0.8%; Fe_2O_3 0.2–0.8% Metal traces (µg/g): Mn Deposits of natural wollastonite typically contain other minerals or contaminants, which may include toxic contaminants, such as crystalline silica and various types of asbestos. Contamination has occurred even in the New York wollastonite deposits, where traces of tremolite were found in a localized area in this deposit.	IARC (1997)

Biopersistence	Experimental	Fibres	Results	References
	Short- and long-term inhalation assay Animals: Wistar rats (male) No. of animals: 3–4 Time of exposure: 5 days (6 h/day) Exposure concentration (mg/m^3): 115 (800 fibres/cm^3)	NYAD-G from NYCO Minerals	Wollastonite fibres were cleared rapidly with a retention half-time of <1 week Mean fibre lengths decreased from 11 µm to 6 µm over a 1-month period, and fibre diameters increased from 0.5 µm to 1.0 µm in the same time	Warheit et al. (1994)
	Intratracheal instillation assay Animals: Wistar rat No. of animals: 5 Dose: 2.0 mg/rat Fibres tested: xonotilite, wollastonite, crocidolite	Four samples (from NYCO) with different mean length and diameter Fibre length range = 1.7–4.3 Fibre diameter range = 0.10–0.85	Estimated half-times for the wollastonite fibres (length <5 µm) ranged from 15 to 21 days	Bellman & Muhle (1994)

Biopersistence	Experimental	Fibres	Results	References
	Intratracheal instillation assay Animals: Wistar rat Dose: 2.0 mg/rat Fibres tested: various coated and uncoated wollastonites	Three sources, including India, Eternit and NYCO Minerals	Estimated half-times for the wollastonite fibres ranged from 10 to 18 days	Muhle et al. (1991, 1994)

Tumour induction	Experimental	Fibres	Results	References
	Intraperitoneal injection assay Animals: Wistar rats Doses: 0.01–0.25 mg/animal Fibres tested: 11 fibrous and 3 granular dusts		Wollastonite fibres were not carcinogenic The authors ascribed the lack of response to wollastonite to its low durability	Pott et al. (1989)
	Intraperitoneal injection assay Animals: Wistar rats No. of animals: 50 Dose: 30 mg/animal Fibres tested: wollastonite and crocidolite	Mean fibre length = 5.6 µm Mean fibre diameter = 0.7 µmf	130 weeks after the start of the treatment, no abdominal tumours were observed among animals treated with wollastonite In a positive control group treated with 3 mg crocidolite, abdominal tumours were observed in 32/50 rats	Muhle et al. (1991); Rittinghausen et al. (1991)
	Intraperitoneal injection assay Animals: F344 rats No. of animals: 13 for each treatment Doses: 5 and 10 mg/rat Fibres tested: chrysotile, glass wool, rock wool, refractory fibres, silicon carbide whisker, titanium oxide (rutile) whisker, wollastonite	Wollastonite from China Mean fibre length = 10.5 µm Mean fibre diameter = 1.0 µmf	After 2 years, no mesotheliomas were found in groups of rats exposed to glass wool, rock wool or wollastonite, but the article is not explicit on this point	Adachi et al. (2001)

Tumour induction	Experimental	Fibres	Results	References
	Long-term inhalation assay Animals: F344 rats No. of animals: 78 Time of exposure: 1 or 2 years (6 h/day, 5 days/week) Exposure concentration: 10 mg/m^3 (360 total fibres/cm^3 or 55 WHO fibres/cm^3)	Mean fibre diameter = 0.1–1.0 µmf	Wollastonite did not cause an increased tumour rate compared with controls The authors ascribed the lack of response to wollastonite to its low biopersistence	McConnell et al. (1991)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Intraperitoneal injection assay Animals: rats Doses: 0.01 and 100 mg/rat Fibres tested: crocidolite, chrysotile, actinolite, erionite, wollastonite, glass fibres 104/475, polypropylene, aramide fibres		After 15–28 months, association of fibrosis and local reactive hyperplasia of partly atypical proliferation of rat omentum mesothelium was observed	Friemann et al. (1990)
	Instillation in the rat lung assay Fibres tested: wollastonite and amosite		Instillation (into the lung) of wollastonite long and short fibres did not significantly influence the cellular parameters studied, in contrast to amosite (particularly the longer fibres)	Hurbánková et al. (1995)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Intratracheal injection assay Animals: rats Dose: 25 mg/rat Dusts tested: crocidolite, quartz, wollastonite	Three wollastonite samples from China (mean fibre length 11.6 µm, mean fibre diameter 1.3 µm) Two samples from NYCO Minerals (mean fibre length 9.2 µm, mean fibre diameter 1.2 µm)	Wollastonite from China induced a significant increase in the indices of fibrogenic effects (total hydroxyproline content, wet weight of the lung, total lipid content in the lung) in comparison with the controls NYAD wollastonite produced only a small but significant increase in hydroxyproline levels The fibrogenicity was considerably less than that of crocidolite and quartz	Cambelova & Juck (1994)
	Intratracheal instillation assay: mesothelial cell proliferation evaluation Animals: Sprague-Dawley rats Fibres tested: wollastonite and UICC crocidolite	NYAD 1250, NYCO Minerals Mean fibre length 1.8 µm, mean fibre diameter 0.44 µm	Intraperitoneal injection of wollastonite fibres induced an early inflammatory and proliferative response that subsided after 21 days In contrast, the number of fibres recovered from tissue digests had not declined 6 months after injection of crocidolite asbestos	Macdonald & Kane (1997)
	Intratracheal instillation assay Animals: Sprague-Dawley rats Dose: 1 mg/animal Fibres tested: Chinese wollastonite and crocidolite	44% fibres >20 µm in length and <1 µm in diameter	After 1 month, the wollastonite-exposed animals developed minimal fibrosis This inflammation did not progress among those animals examined after 3 and 6 months In contrast, after 1 month, the crocidolite-exposed animals developed a very intense multifocal inflammation, which became more intense at 3 and 6 months The authors concluded that wollastonite is less cytotoxic than crocidolite and attributed the "very mild histological reaction" to the short half-time	Tátrai et al. (2004)

Inflammatory response and fibrosis	Experimental	Fibres	Results	References
	Short-term inhalation assay: study of combined effect of cigarette smoke and fibrous mineral Animals: Fischer 344 rats Doses: 30 or 60 mg/m^3, combined with daily exposure to cigarette smoke for 175 days Fibres tested: amosite and wollastonite		Significant rise in binucleated cells after inhalation of wollastonite, but only marginal changes in multinucleated cells and other inflammation parameters Wollastonite, at the same inhalation exposure concentration, caused less toxic effects in rats than amosite	Beno et al. (2005)
	Long-term inhalation assay Animals: F344 rats No. of animals: 78 Time of exposure: 1 or 2 years (6 h/day, 5 days/week) Exposure concentration: 10 mg/m^3 (360 total fibres/cm^3 or 55 WHO fibres/cm^3)		Wollastonite did not induce interstitial fibrosis	McConnell et al. (1991)
	Short-term inhalation assay Animals: rats Time of exposure: 3 or 5 days (6 h/day) Exposure concentration: 50 or 100 mg/m^3 (123 or 835 fibres/cm^3) Fibres tested: wollastonite and crocidolite	NYAD-G from NYCO Minerals	Wollastonite produced transient pulmonary inflammatory responses and increased the enzyme and protein levels in BAL fluid only if the mass median aerodynamic diameter was 2.6 µm and the exposure concentration exceeded 500 fibres/ml The severity and duration of the response to wollastonite were less than those observed with crocidolite	Warheit et al. (1991)

Cytotoxicity	Experimental	Fibres	Results	References

Cytotoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of membranolytic activity Cells: human red blood cells Fibres tested: chrysotile, wollastonite (three varieties)		All wollastonite varieties were found to have smaller haemolytic potential in human red blood cells than that of chrysotile in vitro	Aslam et al. (1995)
	Short-term inhalation assay: study of combined effect of cigarette smoke and fibrous mineral Animals: Fischer 344 rats Doses: 30 or 60 mg/m^3 combined with daily exposure to cigarette smoke for 175 days Fibres tested: amosite and wollastonite		Exposure to wollastonite did not affect cell viability of alveolar macrophages, in contrast to amosite Exposure to wollastonite significantly increased only the cathepsin D activity in BAL cells Smoking significantly enhanced only the effect of amosite	Cerna et al. (2004)

Free radical generation	Experimental	Characteristics of the fibres	Results	References
	In vitro study (fibre-derived radicals): DNA oxidation Fibres tested: crocidolite, glass fibres, potassium titanate		No formation of 8-OH-dG was detected	Nejjari et al. (1993)
	In vitro study: fibre-derived radicals using a cell-free reactive mixture containing deoxyribose and **cell-derived radicals** using PMN suspension	NYAD from NYCO Minerals (length <5 µm)	Wollastonite fibres produced higher ROS levels both in the PMN suspensions and in the cell-free reactive mixtures These ROS were not hydroxyl radicals The authors concluded "that wollastonite fibers are probably less toxic than asbestos fibers"	Governa et al. (1998)

Free radical generation	Experimental	Characteristics of the fibres	Results	References
	In vitro study (cell-derived radicals): measure of release of superoxide Cells: human monocyte-derived macrophage Fibres tested: glass wool, rock wool, micro glass fibre, two types of refractory ceramic fibre, refractory mullite fibre, potassium titanium whisker, silicon carbide whisker, titanium oxide whisker, and wollastonite	Japan Fibrous Material standard reference	Wollastonite did not induce release of superoxide Release of superoxide from macrophages occurs nonspecifically for various types of mineral fibres depending on fibre length	Ohyama et al. (2001)

Genotoxicity	Experimental	Fibres	Results	References
	In vitro study: induction of DNA damage (strand breaks) by the fibres alone or in combination with cigarette smoke Cells: Alveolar macrophages and type II cells isolated from Fischer 344 rats Fibres tested: amosite, wollastonite, rock wool or glass fibres		The number of DNA strand breaks in both cell types was enhanced after exposure to all types of fibre The enhancement was dose dependent The highest level of strand breaks was observed after amosite exposure The combined exposure to mineral fibres and cigarette smoke showed synergic effects on the level of the strand breaks	Kovacikova et al. (2004)

Apoptosis	Experimental	Fibres	Results	References

Apoptosis	Experimental	Fibres	Results	References
	In vitro study Cells: Pleural mesothelial cells (rabbit or human) (1–10 µg/cm^2) Fibre concentration: 1–10 µg/cm^2 Fibres tested: crocidolite, amosite, chrysotile and wollastonite		Wollastanite did not induce apoptosis compared with control	Broaddus et al. (1996)
	In vitro study Cells: Pleural mesothelial cells (rabbit) Fibre concentration: 1–10 µg/cm^2 Fibres tested: crocidolite, and wollastonite as a control		Wollastanite did not induce apoptosis compared with control	Marchi et al. (2000)
	Intrapleural instillation Animals: rabbits Fibres tested: crocidolite, and wollastonite as a control		Wollastanite did not induce apoptosis of mesothelial cells compared with control	Marchi et al. (2000)

End-point	Fibre modification	Experimental conditions	Results	References
Fibre dimensions				
Deposition of inhaled fibres				
Chemical composition				
Free radical generation				
Dissolution				
Clearance and biopersistence		Inhalation study in rats for 5 days (800 fibres/cm^3, 115 mg/m^3)	Rapid clearance from the lung (half-life: <1 week)	Warheit et al. (1999)
Fibre fragmentation in situ				

End-point	Fibre modification	Experimental conditions	Results	References
Modes of action of fibres in pulmonary/pleural carcinogenesis				
Genotoxicity				
Malignant transformation, alterations in growth kinetics, inhibition of differentiation				
Cell proliferation				
Chronic inflammation, release of cytokines, growth factors, reactive species		In vivo study in rats	Wollastonite induced mild pulmonary interstitial fibrosis The effects caused by wollastonite were mild compared with those of crocidolite	Tatrai et al. (2004)
Short-term animal tests				
Carcinogenicity studies		Carcinogenicity in rats, intraperitoneal injection	No tumours	Pott et al. (1987, 1989)

Table 2.16 Physicochemical properties, biopersistence data and mechanistic data for xonotlite

	Xonotlite	References
Fibre dimensions and surface area	Fibre diameter, µm = 0.1–0.5 Fiber mean length, µm: 1.5; range = 1.0–5.0 Aspect ratio = 10 Powder: single crystals of acicular morphology and of agglomerates of these crystals Surface area (BET) = 30–60 g/m^2	UBE industries
Chemical composition	6CaO $6SiO_2$ H_2O manufactured by hydrothermal synthesis from hydrated lime and silica Chemical analysis: SiO_2 45–49%; CaO 42.0–43.5%; MgO 0.1–0.23%; Al_2O_3 0.15–1.15%; Fe_2O_3 0.11% Metal traces (µg/g): Sr (177); Mn (21); Pb(9.5); Cd (2)	UBE industries
Dissolution	Solubility in water: 0.03 g/l; Gamble's solution: 0.24 g/l	UBE industries

Biopersistence	Experimental	Fibres	Results	References
	Intratracheal instillation assay Animals: Wistar rat No. of animals: 5 Dose: 2.0 mg/rat Fibres tested: xonotlite, wollastonite, crocidolite	Single crystals of acicular morphology with a median length of 1.3 µm	More than 99% of single crystals and about 85–89% of the agglomerates were eliminated 2 days after instillation	Bellman & Muhle (1994)

Inflammatory response	Experimental	Fibres	Results	References

Inflammatory response	Experimental	Fibres	Results	References
	Intratracheal instillation Animals: rats No. of animals: 5 for each treatment Doses: 1, 5 and 10 mg/rat Fibres tested: UICC chrysotile B asbestos, short chrysotile 4T30, attapulgite, xonotlite and Fiberfrax (an aluminium silicate)	Fibre mean diameter, μm = 0.2 Fibre mean length, μm: 1.2; range = 1.0–5.0 Aspect ratio = 6	Xonotlite caused minimal inflammatory reactions detectable only at high dose (10 mg) and by bronchoalveolar analysis (chrysotile at all doses tested induced fibrotic lesions and inflammatory response)	Lemaire et al. (1989)

Cytotoxicity	Experimental	Fibres	Results	References
	In vitro study: measure of membranolytic and cytotoxic activity Cells: rat pulmonary alveolar macrophages Fibre concentrations: 50 and 250 μg/ml Fibres tested: chrysotile, attapulgite clay, Fiberfrax (an aluminium silicate) and xonotlite	Fibre mean diameter, μm = 0.2 Fibre mean length, μm: 1.2; range = 1.0–5.0 Aspect ratio = 6	At 50 μg, cytotoxic effect was in the decreasing order: Fiberfrax > attapulgite > chrysotile > xonotlite At 250 μg: all four silicates were equivalent Xonotlite had a strong haemolysis potential, very close to that of chrysotile	Nadeau et al. (1987)

Free radical generation	Experimental	Fibres	Results	References
	In vitro study (fibre-derived radicals): DNA oxidation Fibres tested: crocidolite, glass fibres, potassium titanate, xonotlite		No formation of 8-OH-dG except for crocidolite	Nejjari et al. (1993)

Genotoxicity	Experimental	Fibres	Results	References
	In vitro study: induction of UDS Cells: rat hepatocytes in primary culture Fibre concentrations: 1 and 10 µg/ml Fibres tested: attapulgite, xonotlite, sepiolite		None of the fibres showed detectable UDS-eliciting activity	Denizeau et al. (1985)