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Mechanisms of Fibre Carcinogenesis

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CONSENSUS REPORT

This Consensus Report was prepared by a group of experts convened at IARC on 9–11 January 1996. The first part of the report addresses the strengths, weaknesses and gaps in the present knowledge on fibre characterization, genotoxicity, cell proliferation and activation, and animal studies. The second part of the report provides answers to specific questions on the relevance of mechanistic data from in-vitro and in-vivo assays in the assessment of the carcinogenic risk of fibres to humans. Finally, the relevance of mechanistic data in the evaluation of fibre carcinogenicity is discussed.

Fibre characterization

Irrespective of study type or design, the full characterization of all particulate material in a test sample is an essential step in the understanding of the mechanisms of fibre carcinogenesis. There is a broad consensus on many of the parameters necessary for fibre characterization and on the methodology needed to obtain these data.

In terms of the form of the test material, the dimensions of all particles (fibrous and non-fibrous) are important, and are best expressed as bivariate size distributions, i.e. diameter (which primarily determines respirability) and length (which is related to biological activity). Methods of fibre characterization range from relatively simple procedures (such as the use of optical or electron microscopy), to highly specialized techniques (such as laser-assisted microprobe mass spectrometry analysis or LAMMA). Aspect ratios (length:diameter) and size-specific fractions have both biological and regulatory significance, and measurement of dimensions is best accomplished by transmission electron microscopy (TEM). Scanning electron microscopy (SEM) can be equally effective in cases where its magnification and contrast are adequate for the fibre type(s) in question. Micromorphology, measurable by TEM but rarely reported for samples used in biological systems, consists of steps, kinks, edges, etc., and contributes to both reactivity and durability. Specific particle surface area may also be measured using low-temperature nitrogen adsorption. Crystallinity can be evaluated by X-ray diffraction or by selected area electron diffraction (SAED). Although crystallinity is of most importance in relation to mineral fibres, some ceramic materials are crystalline and at least one – silicon carbide 'whiskers' – shows biological effects in both animals (cytotoxicity) and humans (fibrosis).

All aspects of the chemical composition of test materials are important, including the intrinsic chemical constituents of fibres, their surface chemistry and the identity of any chemicals derived from contaminants or acquired during storage. One example of chemical composition that has potential mechanistic importance is iron content. Iron is particularly important in the generation of reactive oxygen/nitrogen species (ROS) at the surface of asbestos fibres. In amphiboles (such as amosite and crocidolite), iron is an intrinsic constitutive component, and this may be significant in view of the generally observed greater carcinogenic potential of these fibre types in humans. However, iron may also be present on other fibre types, for example substituting for magnesium ions in chrysotile. This is a highly variable phenomenon that may vary from sample to sample. Iron may also be present in man-made fibres, as a constituent or as an impurity. Which type(s) of iron, if any, have importance for carcinogenicity via ROS-related mechanisms is unknown; there does not appear to be a dose-relationship between iron content and the generation of ROS.

ROS generation at the solid–liquid interface can be measured by spin trapping or by secondary biochemical reactions, such as DNA damage or lipid peroxidation.

Ferrous iron and other ions are exposed in milling, and asbestos minerals may be activated or otherwise altered: ROS release, among other effects, is modified. Metal contamination of fibres has been reported from the milling device.

The importance of biopersistence is well recognized and was the subject of a recent IARC/INSERM/CNRS symposium (see Bignon *et al.*, 1994). Reingestion cycles for macrophages may also be important. Biopersistence studies *in vivo* (animals and humans) and the more recently developed assays of fibre durability in cellular and acellular in-vitro studies should now be a routine part of particle characterization.

Strengths, weaknesses and gaps in fibre characterization

There is a large and historically useful literature on many aspects of fibre characterization. This is a multidisciplinary field based on industrial hygiene, physical and inorganic chemistry, inhalation toxicology, experimental pathology and mineralogy. Much information is available on the measurement of fibre characteristics and there is a considerable data bank extending back for many years. Investigators of new biological phenomena must be aware of this and also be familiar with the fibre characterization techniques described above and in the authored paper by B. Fubini in this volume. New techniques, such as atomic force microscopy, have emerged in the last five years, and investigators should be aware of these.

While it is acknowledged that crystallinity and micromorphology have important effects on fibre reactivity and durability, these parameters are rarely specified in sample characterization for biological studies. There are also few measurements available on specific particle surface area.

Data have been lacking on a number of chemical parameters, including (but not limited to) adsorptive capacity for both exogenous and endogenous materials. There is also a need for the analysis of bulk composition (full chemical analysis) and surface composition (including redox states of ions). Particle chemistry has traditionally been measured by electron diffraction (ED) analysis coupled with TEM or SEM, but these methods give only a proportional chemical make-up, mainly of the particle surface and the subsurface layers. There are some data on exogenous and endogenous adsorbed materials and on the many other chemical parameters that should also be measured. These data are widely available, although interlaboratory comparisons are difficult due to different techniques of sample preparation and analysis.

There remains taxonomic confusion and lack of standardized operating definitions for fibres. 'Asbestos' is often inappropriately used as a generic, homogeneous rubric, and even when an asbestos fibre type is specified, its source is rarely stated. Even standardized samples often contain mixtures of fibres. For example, the 'Union Internationale Contre le Cancer (UICC) B' chrysotile sample is a blend from eight different sources, which are known to have differences in human disease potential. Terms such as 'asbestiform' and even 'fibre' are highly controversial across disciplines; for example, most mineralogists would not consider particles having an aspect ratio of less than 10:1 as 'fibrous'. In this report, however, the WHO definition of a fibre (aspect ratio greater than 3:1) is used (WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF, 1985). The definition of what constitutes 'long' or 'short' fibres is also debatable. However, this may not be a problem if bivariate size distributions are obtained and 'biological operational definitions' are used; for example, an important criterion of fibre length may be based on the species-specific ability of macrophages to phagocytize a particular fibre completely. There needs to be agreement as to the best expression of fibre 'exposures' in in-vitro and in-vivo systems. Numerators consisting of fibre numbers or surface area are preferable to fibre mass.

There is more unanimity on the physical characteristics of man-made vitreous fibres (MMVF) and other man-made fibres. A serious question must be asked as to whether 'asbestos' or other natural fibres can be as well characterized for biological studies as man-made fibres – certainly the task is more difficult.

The chemical make-up of most mineral fibre samples has not been adequately characterized. Iron content, for example, can be in the form of an endogenous coating, as in asbestos bodies, and the surface composition may not reflect the constituent chemical composition. Furthermore, the type of iron or other transition elements on or in a fibre that are potentially toxic is also unknown. Quantitation of ROS present in culture media is not usually performed. Whether natural or man-made, inorganic and organic (e.g. cellulose, *para*-aramid) fibres should be fully characterized by dimensions, chemistry and biopersistence when used in any well-conducted biological study. The following information should also be provided: time and place of acquisition (for example, mine coordinates, geological site, workplace sample, etc.); contaminants (mineral and chemical); and storage conditions (time of storage, effects of storage solutions on particle chemistry and possible oxidative changes, possible adsorption from containers). The potential for human exposure to the fibre sample should also be stated. Fibre characterization should be performed before, during, and at the end of experiments.

Genotoxicity, cell proliferation and cell activation

Carcinogenesis by fibres appears to be a multistage process and may arise by the ability of fibres to cause (i) altered expression or function of key genes arising from genetic or epigenetic alterations; (ii) altered cell proliferation; (iii) altered regulation of apoptosis; or (iv) chronic, persistent inflammation.

In the lung, multiple cell types have been shown to proliferate in response to deposited fibres, including those that are not targets for neoplastic change by fibres. Therefore, additional mutagenic and/or clastogenic events are required for the development of tumours. From in-vitro cell assays, all asbestos fibres tested have demonstrated potential genotoxicity in mammalian cells. From studies with asbestos, it is clear that these fibres can activate macrophages and epithelial cells to release inflammatory mediators, cytokines and growth factors that may alter epithelial and mesothelial cell proliferation and differentiation. Recent data have shown the following: (i) fibres can bind to the plasma membrane and activate cells; and (ii) asbestos fibres can activate multiple intracellular signalling pathways and transcription factors. Oxidative stress may be central to the fibre-mediated activation of key inflammatory/proliferative signalling pathways via redox-sensitive transcription factors such as nuclear factor κ B (NFκ B) and the activator protein AP1.

Strengths, weaknesses and gaps in genotoxicity studies

Genotoxicity studies have provided mechanistic information at the cellular and molecular level. Cytogenetic studies have suggested that chromosomal aberrations, deletions and aneuploidy are relevant for fibre carcinogenesis. There is a fairly good consensus between these types of studies, which have been carried out with different types of mammalian cells.

The major weakness of these in-vitro genotoxicity studies is lack of validation *in vivo*. In addition, the total data bank on fibres is small and only a few fibre types have been tested. The influence of fibre durability has not yet been assessed in in-vitro genotoxicity assays. In addition, the following shortcomings in experimental design can be identified in many of the in-vitro cell studies: an absence of adequate statistical analysis; a lack of positive and negative controls; and the potentiation of DNA damage caused by either exogenous components (e.g. iron) or cell-derived components (e.g. oxidized lipids) in the culture media.

Limited data are available on the formation of oxidized bases (e.g. 8-hydroxydeoxyguanosine, 8-OHdG) in in-vitro cell models. The specific oxidizing agent (hydroxyl radical, OHº ; peroxynitrite; lipid hydroperoxides) or clastogenic factors have not been identified.

Strengths, weaknesses and data gaps in cell proliferation and activation studies

A variety of target cells proliferate following short-term in-vivo exposure to fibrous materials; these observations are consistent with fibre-induced disease outcomes in other studies. Specifically, long crocidolite asbestos fibres induce greater lung epithelial and/or mesothelial cell proliferation in intratracheally and intraperitoneally exposed rats than do short fibres. Similarly, in agreement with tumour outcome in inhalation bioassays, ceramic-fibre-exposed hamsters show greater mesothelial cell proliferation than do rats. Lastly, in rats, mesothelial cell proliferation following ceramic fibre exposure is greater than that induced by glass fibre in apparent correlation with tumour outcome in recent chronic inhalation studies.

Studies using cells exposed to fibres *in vitro* have demonstrated fibre-specific activation, which correlates with the pathological responses observed *in vivo*. For example, cytokine and oxidant release by macrophages *in vitro* is greater in response to long amphibole asbestos than to a short fibre preparation obtained from the same material. In addition, cytokine release from pulmonary epithelial cells was greater in crocidolite-exposed cells than in glass-fibre-exposed cells.

There are multiple weaknesses in the published in-vitro cellular assays. In general, there is a poor characterization of dose with regard to fibre number, dimensions and surface area; also, the specific dose internalized by the target cells is rarely determined. The lung environment modifies the surface of fibres by coating them with endogenous molecules before they make contact with cells; this is seldom addressed in in-vitro studies. Cell lines are highly selected and therefore it is uncertain whether findings obtained with them are representative of target cells *in vivo*. Stable cultures of mesothelial cells are difficult to obtain for in-vitro studies. Most assays are based on the response of the whole cell population; since cells are known to be heterogeneous, methods that allow determination of response in individual cells are desirable. There is limited standardization between laboratories in the assessment of methods and this precludes critical evaluation of differences in the effects reported; for example, there is limited standardization in culture conditions and media composition, including the levels of serum and growth factors. There is a need for in-vivo validation and confirmation of in-vitro findings.

In short-term in-vivo studies, intratracheal instillation and intracavity injection are widely used methods of administration, but the results can be difficult to interpret because of high-dose exposures that may result in uneven deposition. Much of the data bank on the rodent mesothelioma cell and its molecular biology comes from tumours produced in animals exposed by intraperitoneal or intrapleural instillation.

The mechanism or combinations of mechanisms that underlie the epithelial and mesothelial cell proliferative response to fibres are unclear. It is not known whether there are multiple fibre-specific and cell-specific activation pathways or a common mechanism of cell activation. The relationship between cell proliferation in different asbestos-related diseases and in-vivo genotoxic effects is unknown. Likewise, the mechanisms responsible for proliferative responses in short- versus long-term exposures and to low- versus high-dose exposures remain to be elucidated. It is premature to derive generalizations about mechanisms because studies have been limited to a restricted number of fibres. In many cases there has not been a wide enough dose–response relationship demonstrated to allow comparisons between fibre types and preparations to be made.

Fibres may stimulate cell proliferation by several pathways: (i) activation of intracellular signalling pathways mimicking growth factors; (ii) stimulation of growth factor production; and (iii) up-regulation of growth factor receptor expression. Information on defining the receptors that fibres interact with at the cell surface is limited, and the data that are available are confined largely to macrophages exposed to asbestos. There is also limited information on receptors for fibres on epithelial cells and mesothelial cells and for man-made fibres in general.

Qualitative or quantitative differences in the proliferative response of rodent mesothelial cells have been reported, but it is unclear how these correlate with mesothelioma induction. Since a variety of soluble lung toxins can cause mesothelial and epithelial proliferation, there is a gap in the knowledge regarding the mechanism of fibre-specific proliferation. This gap extends to the cytokinetics of epithelial and mesothelial cells and the factors that lead to sustained proliferation in the case of fibres. There is a particular gap in the knowledge concerning the responses of the parietal pleural mesothelium. Limited information is available on the ability of fibres other than asbestos to cause cell activation and proliferation.

In mechanistic terms, little is known of the relationship between fibrosis and neoplasia, although the inflammatory fibroproliferative environment may promote neoplasia because of the localized accumulation of mitogenic mediators from activated inflammatory cells and fibroblasts.

There is a gap in the knowledge of the relative importance of fibre-derived and cell-derived ROS and their interaction in causing oxidative stress. There is a need for data to fill the gap on the adaptive responses of fibre-exposed tissue including induction of antioxidant defences and the impact of fibre-mediated oxidative stress on the redox state of target cells.

In using markers of proto-oncogene and tumour suppressor gene alteration in trying to understand the mechanism of mesotheliorna induction, the use of cell lines and mature tumours may be a limitation because important events may have occurred early *in situ*. Time-course studies of the lesions *in situ* are necessary to investigate these early changes.

Animal studies

All animal studies with fibres should be designed and conducted to give exposure–dose–response relationships using more than two exposure–dose levels, with the highest dose being at the maximal tolerated dose (MTD). There is, however, no consensus on how the MTD should be determined in a range of experimental situations. An initial definition of an MTD that has been used for long-term carcinogenicity studies is 'a dose that produces no increased mortality compared to controls, no shortening of life span other than that resulting from tumour development and no more than a 10% weight gain reduction compared to controls'. This definition, however, is not adequate for all types of studies, and the MTD might have to be defined differently according to the exact processes examined. In studies on fibres, indicators of MTD that might be of widespread usefulness include the following, where applicable: increases in inflamatory parameters; increased target cell proliferation; altered histopathology other than carcinogenicity; prolonged lung clearance function; and the existence of non-linear fibre retention kinetics. In most cases, several of these indicators should be considered. However, the magnitude of change in each case has not yet been determined.

Strengths, weaknesses and data gaps in animal studies

Weaknesses of both short-term and long-term animal studies that can be specified from existing publications include inadequate details of techniques used and most particularly inadequate quantification of the fibres used. The exact details of fibre length and diameter (bivariate analysis) should always be recorded as well as the amount of non-fibrous particulate material in the dust specimens. In addition, most studies published so far lack information on dose response, a most important aspect.

The major strengths of short-term studies are that, because they are both shorter and less expensive than long-term studies, a wide range of materials can be examined at one time. At present, they are mainly used to examine mechanisms of fibre–cell interactions. These studies should not be limited to short-term work, since one of their main weaknesses at present is that mechanisms are studied for a few days when information on the longevity of the process is needed. Many short-term results are suggested as predictors (biomarkers) of long-term pathological change, but, as yet, there have been few confirmations from long-term studies that these events actually occur. This is one of the major gaps that needs to be filled before we can evaluate fully short-term mechanistic studies.

Long-term studies can be used to demonstrate that mechanistic changes first examined in short-term tests are important throughout actual disease development. At present there is no consensus on the best animal species to mimic and predict human reactions. The rat is most commonly used in chronic inhalation studies but there are data suggesting that the hamster may be better for studies on mesothelioma development. A multidose asbestos inhalation study in rats and hamsters is needed, although, in future, other species may be considered; a well-characterized amphibole asbestos, a known human carcinogen, should be used. Such a study is not available at present. This circumstance was identified as a significant gap. Weaknesses and gaps in studies so far published relate mainly to the general considerations of inadequate fibre characterization but also to the lack of a full examination of fibre retention kinetics and biodurability and the processes that affect these. Another gap relates to the use of cells and lung tissues from animals exposed *in vivo*, preferably by inhalation, for subsequent in-vitro mechanistic studies. As an example, a technique for isolation of type II alveolar epithelial cells for evaluation of specific mutations has been developed only recently.

A major debate concerning long-term in-vivo studies relates to the suitability of the methods of fibre administration, with the three main techniques being inhalation, intratracheal instillation and intracavity injection. Inhalation is suggested as being a natural method of exposure where the normal lung defences are operating. It permits an examination of biopersistence, pulmonary toxicity, fibrosis and carcinogenicity with relevance to both pulmonary carcinomas and mesotheliomas, although it should be remembered that the dimensions of respirable fibres are different between rats and humans. Intratracheal injection has the disadvantage of being an artificial exposure that can swamp normal lung defences. However, if this is accepted, the technique may be used to examine biopersistence, pulmonary toxicity and carcinogenicity. Intracavity injection is also an artificial method of administration that bypasses lung defences, and there is a limit to the maximal diameter of the fibres that can be studied. However, the technique may be of use to examine the mechanisms of development of mesotheliomas and the long-term persistence of fibres in these cavities compared to the lung. This has been little examined and is a major gap in our knowledge.

The major point of difference regarding the value of inhalation versus injection studies relates to their sensitivity and the applicability of results for predicting human hazard and risk. The point of major importance with respect to mechanisms of fibre carcinogenicity relates to the issue of dose, as mechanisms at high-dose levels may be different from those at low-dose levels. Although all techniques may be of use in examining particular aspects of the carcinogenic process from a mechanistic point of view, it needs to be considered that, after deposition in the respiratory tract, translocation of fibres from the alveolar region to the pleura represents a selection process in terms of fibre dose and fibre size. This aspect is circumvented with direct intracavity administration of fibres.

With regard to the development of fibre-related disease and tumour production in the presence of other non-fibrous dusts, chemical carcinogens, viruses and radiation, our knowledge of the effects of combinations such as these is almost one large gap. The few studies on these combinations that have been published have major weaknesses in study design, particularly their lack of proper controls. What is needed are a series of studies where fibres are administered with and without one of the materials under consideration in controlled multidose studies using a protocol where some level of fibre carcinogenicity is known to occur and including the collection of data on variations of fibre clearance or fibre durability that might occur with the different combinations.

Relevance of in-vitro assays

To what extent can physico-chemical properties be used to predict potential carcinogenicity of fibres?

At present, there is insufficient understanding of how the physical and chemical properties of fibres contribute to mechanisms of fibre-induced carcinogenesis to make reliable predictions of the carcinogenic potential of fibres based solely on these types of data. In this respect, it was considered that there were no combinations of physical and chemical data that could be used to identify a fibre as a carcinogen or a noncarcinogen. However, there are physical and chemical properties of fibres that have been associated with fibre toxicity *in vitro* and toxicity and/or carcinogenicity *in vivo*. In this respect, characterizing selected physical and chemical properties of fibres could be useful in the context of screening assays to make inferences on the relative potential of fibres to produce adverse effects *in vivo*. Given the current limitations of in-vitro fibre testing (see above), these inferences would need to be validated *in vivo*.

Fibre dose, dimensions and durability are currently accepted as important parameters; are there other important characteristics relevant to potential bioactivity?

In addition to dimension and durability, there may be other aspects of the physical and chemical properties of fibres that can provide information on potential fibre toxicity *in vivo*. These were considered to include the following: the presence of iron or other transition metals on fibres; the ability of a fibre to accumulate iron; the ability of fibres to generate free radicals; the ability of a fibre to interact with and alter biologically relevant molecules (e.g. DNA, lipids, proteins); and the ability of fibres to cause lysis of erythrocytes/liposomes. In addition to these endpoints, information on the ability of fibres to activate cells *in vitro* (e.g. to produce ROS and cytokines and/or alter the expression of proliferation-related genes of macrophages, epithelial cells, mesothelial cells and other relevant cells) may provide insights into the relative potential of fibres to elicit adverse effects *in vivo*. Since the precise relationships between these various aspects of fibre activity and potential chronic toxicity *in vivo* are incompletely understood, these data would be useful primarily in the context of screening assays and the need for chronic toxicity to be confirmed by appropriate in-vivo testing.

Are in-vitro genotoxicity assays relevant to fibre carcinogenesis?

It is generally agreed that genetic alterations play a critical role in the carcinogenic process. Thus, conceptually, well-designed and validated genotoxicity assays can provide information for assessing the potential carcinogenicity of materials.

In the context of genotoxicity testing of fibres, current in-vitro genotoxicity tests possess limitations common to all current in-vitro assays on fibres. In addition, questions exist regarding proper validation of genotoxicity tests for fibres in that there are no clear negative control materials, nor is there agreement against which in-vivo carcinogenicity data the tests should be compared. Recognizing these limitations, however, in-vitro genotoxicity assays, particularly those assessing cytogenetic effects, do provide some information on potential in-vivo genotoxicity of fibres and, in this regard, potential carcinogenicity. However, given the current limitations of in-vitro testing methods for fibres, this type of in-vitro information should be validated by appropriate in-vivo tests.

What is the relationship between acute in-vitro effects of fibres on growth factor and proto-oncogene expression and chronic persistent proliferation of target cell populations?

Fibres can persist within the lung, and studies have demonstrated fibre-specific differences in biopersistence. Conceptually, the biopersistence of fibres should relate to their ability to activate gene expression and induce cell proliferation over extended time periods. However, there are significant gaps in our knowledge of the relationships between growth factor/proto-oncogene expression in in-vitro or short-term in-vivo studies and cell proliferation. In addition, there are gaps in our understanding of how the effects of fibres on cell proliferation after acute exposure relate to cell proliferation after chronic exposure. Therefore, at present, the relevance of in-vitro and acute in-vivo changes in gene expression to potential chronic proliferative effects is uncertain.

Relevance of in-vivo assays

Can the genotoxic effects of fibres be assessed in vivo?

To date, there has been virtually no assessment of genotoxicity *in vivo*. In theory, it should be possible using such endpoints as unscheduled DNA synthesis, the occurrence of mutations *in vivo* and also the occurrence of cytogenetic abnormalities in target cell populations following exposure to fibres.

It will be technically difficult to undertake such studies with mesothelial cells due to the mono-layer nature of the mesothelium and the associated difficulty in isolating sufficient numbers of these cells. For other target cells, validated separation techniques already exist (e.g. type II alveolar epithelial cells).

What are the links between inflammation, fibrosis and cancer induced by fibres?

Experimental studies with fibres showing significant numbers of lung tumours have always shown high levels of pulmonary fibrosis. This does not necessarily indicate a cause–effect relationship since both processes may be a response to high fibre doses. Intracavity injection studies using high doses result in both mesotheliomas and fibrosis. There is, however, debate concerning whether low but carcinogenic doses of fibres also result in fibrosis in this model. This may be due to the lack of sensitive methods for estimating fibrosis, particularly in the peritoneal cavity. The relationship between pleural fibrosis and mesothelioma has not been determined. It is not known whether malignant mesothelioma arises from the visceral pleura, the parietal pleura or both.

Fibre-induced chronic inflammation leads to fibrosis. There are no data on direct links between inflammation and carcinogenesis. However, one widely held theory is that, in areas of chronic inflammation, substances such as ROS and cytokines are produced that may be involved in tumour production.

Do *lung burden and biopersistence of fibres in animals reflect lung burden and biopersistence of fibres in humans?*

Sizes of 'respirable' fibres are different between rats and humans. This means that, for any dust cloud, penetration and deposition (and therefore lung burden accumulation) will be different. Once in the lung, differences in the clearance and translocation rates will also affect the retained lung burden. Few data exist on these matters, although human alveolar macrophage-mediated clearance of non-fibrous materials is slower than that for rats. The rates for clearance from the interstitium have not been compared. Fibre dissolution, which is a chemical process, may not differ substantially between species, although some work with cobalt oxide does show some interspecies differences.

Is total lung fibre burden an accurate assessment of fibre disposition or are there localized areas of fibre deposition and retention that correlate with the development of bronchogenic carcinoma and mesothelioma?

Localized accumulation of fibres in the form of fibre-containing lesions at the bifurcations of terminal and respiratory bronchioles certainly occur. Other possible 'hot spots' are areas of interstitial or peribronchiolar fibrosis and lymphatic stomata in the parietal pleura. However, no data exist as to whether dissolution or clearance from these sites of aggregation differs from the rest of the lung. In rats, pulmonary tumours often appear to develop in the proximity of 'hot spot' lesions. However, human bronchogenic carcinomas develop mainly in the larger proximal bronchial airways and not in the distal lung parenchyma.

Does the inhalation of fibres or mixed fibres and non-fibrous dusts impair clearance in rats? Is this mechanism relevant for humans?

Inhalation of fibres at high doses in experimental studies has been shown to produce an increased rate of accumulation in proportion to the mass of dust deposited. Information is lacking on the effects that mixtures containing non-fibrous dusts have on the accumulation or clearance of fibres. Fibres, themselves, have been shown to impair the clearance of some materials such as cobalt oxide at moderate doses.

It is not certain whether human asbestos exposures in the past were ever sufficiently high to produce impaired clearance.

Relevance of mechanistic data in evaluation of fibre carcinogenicity to humans

Cellular and molecular mechanisms of fibre carcinogenesis

The exact mechanisms leading to the development of cancer after exposure to asbestos fibres are poorly understood. Most lung cancers in humans exposed to asbestos occur in cigarette smokers; however, an excess of lung cancer also occurs in a small percentage of people exposed to asbestos fibres alone. It is not known whether the same mechanism is responsible for the development of these tumours in smokers and nonsmokers exposed to asbestos. While fibres can produce both lung cancer and mesothelioma, different patterns of molecular alterations have been identified in human lung cancers associated with asbestos exposure and cigarette smoking in comparison with diffuse malignant mesothelioma. Therefore, it is possible that different cellular and molecular mechanisms are involved in the development of these two tumour types. Specific molecular alterations unique to asbestos-induced tumours have not been identified in either humans or experimental animals. Therefore, it is difficult to assess whether similar molecular mechanisms are responsible for the development of lung cancer and mesothelioma in humans and rodents exposed to asbestos fibres. Similarly, it is not known whether different types of carcinogenic fibres activate common or different mechanistic pathways.

As summarized in the paper by A.B. Kane in this volume, five mechanistic hypotheses for fibre carcinogenicity have been proposed:

– Fibres generate free radicals that damage DNA.

– Fibres interfere physically with mitosis.

– Fibres stimulate proliferation of target cells.

– Fibres provoke a chronic inflammatory reaction leading to prolonged release of ROS, cytokines and growth factors.

– Fibres act as co-carcinogens or carriers of chemical carcinogens to the target tissue.

This Consensus Report has summarized the strengths, weaknesses and gaps in the published data that are relevant for these hypotheses; these observations are summarized in **Table 1**. It should be noted that some of the experimental endpoints listed in this table have also been noted after exposure to non-fibrous particles. These experimental observations reveal associations between exposure to asbestos fibres and specific endpoints in in-vitro or in-vivo models. Some of these associations have also been observed in people exposed to asbestos fibres. However, few experiments have been conducted to assess critically the causal relationship between these changes and the development of lung cancer or mesothelioma.

The inflammatory endpoints measured in various in-vitro and in-vivo assays are especially pronounced at high-dose exposures. It is not known whether tumours produced by high-dose exposures develop via similar or different mechanisms in comparison with low-dose exposures. Most in-vitro studies have been conducted at relatively high fibre:cell ratios; the relevance of these data for chronic exposures at lower doses *in vivo* is also questionable.

Overall, the available evidence in favour of or against any of these mechanisms leading to the development of lung cancer and mesothelioma in either animals or humans is evaluated as weak.

Recommended experimental studies

Future evaluations of fibre carcinogenicity where human epidemiological data or chronic inhalation assays are limited or not available will depend in part on mechanistic information based on relevant experimental models. The Workshop concluded that the following experimental studies would provide additional data for future evaluation of fibres:

A multidose, chronic inhalation study in rats and hamsters using a well-characterized amphibole sample; this study should include relevant short-term endpoints or biomarkers that could be evaluated in future mechanistic studies.

New in-vitro models including development of systems to evaluate the effects of fibre dissolution and in-vivo/ex-vivo assay systems, especially for evaluation of the potential genotoxic and clastogenic effects of fibres.

References

Bignon, J., Saracci, R. & Touray, J.-G, eds (1994) *Biopersistence of Respirable Synthetic Fibres and Minerals.* (*Environ. Health Perspectives*, Vol. 102, Suppl. 5)

WHO/EURO Technical Committee for Monitoring and Evaluating Airborne MMMF (1985) *Reference Methods for Measuring Airborne Man-made Mineral Fibres (MMMF)*, Copenhagen, World Health Organization Regional Office for Europe

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Table 1. Summary of experimental endpoints after in-vitro and in-vivo exposure to fibres

Experimental design	Oxidant-induced damage	Aneuploidy	Cell proliferation	Inflammation	Co-carcino-genicity
In vitro					
Rodent cell lines	++	++	+/–	++	+/–
Human cell lines	+	+/–	0	++	0
In vivo					
Rodents - short term	+	0	++	++	0
Rodents - long term	0	0	++	++	+/–
Humans	0	0	0	++	+/–

++, strong effect; +, weak effect; –, no effect; 0, no data; +/–, contradictory data.
See authored papers elsewhere in this volume for a full discussion of these experimental data.