



Review of FIBRE TOXICOLOGY



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HSE BOOKS



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CONTENTS

Introduction v

Summary 1

Property and uses of filters 5

Human health effects from exposure to asbestos 8

Deposition and clearance 17

Animal studies in fibre toxicity 24

Mechanisms of fibre toxicity 42

Fibre toxicity testing strategy 50

Relationship between fibre size and toxicity 55

References 62

Abbreviations 72

INTRODUCTION

This review aims to present the HSE stance on fibre toxicology. The primary objective is to provide a scientific framework within which fibre toxicology data can be interpreted. This is needed in order to develop a reliable basis for identifying the health hazards and predicting the dose-response characteristics of new and existing fibres. One of the main purposes of this document is to provide a rational basis for evaluating evidence derived from animal studies with fibrous substances, particularly for those fibres which have limited toxicological databases, often with no worthwhile human data.

A further aim of this review is to summarise recent views and evidence relating to the dose-response relationships for the main asbestos-related diseases. This is because the toxicological and epidemiological evidence with asbestos can serve as a benchmark in evaluating the human health hazards of other fibres. However, it is not the purpose of this review to provide a comprehensive coverage of asbestos epidemiology.

It should be noted that this review does not address the question of the tendency of fibres to become airborne, ie dustiness. Although undoubtedly an important property, the position has been taken that dustiness is an occupational hygiene issue, influencing exposure and hence risk; this review focuses more on those properties of fibres that influence their toxicological hazard.

Given the vast literature available in the field of fibre toxicology this review is not based entirely on primary literature sources, but has made use where possible of recent 'state of the art' reviews.

SUMMARY

This review starts with an assessment of the current position regarding the human health data for asbestos, as this is where much occupational health attention on fibres has been focussed. The document then moves on to consider more general issues in fibre toxicology.

Human health effects of exposure to asbestos

All forms of asbestos may cause pulmonary fibrosis, lung cancer and mesothelioma, but the degree of hazard depends on fibre type (greater with amphiboles than with chrysotile) and on the fibre size distribution (long fibres more hazardous than short). From this it is apparent that meaningful comparisons of the incidence of disease observed in different occupational cohorts should take careful account of differences not just in fibre type, but also in the airborne fibre size distributions.

It is concluded that there will be a threshold level of exposure below which no radiological or clinical manifestation of pulmonary fibrosis (asbestosis) will occur. The value for the threshold, and indeed the slope of the dose response curve, depends on fibre type and the fibre size-distribution in the workplace.

There appears to be an association between pulmonary fibrosis and lung cancer in that both diseases show a similar dose-response relationship with respect to asbestos exposure, show similar latent periods for development, show a similar dependence on fibre type and size, and both diseases emanate from the same underlying chronic inflammatory condition. These observations suggest that asbestos-induced lung cancer, like fibrosis, is a threshold phenomenon. It can be concluded that exposures which are insufficient to elicit chronic inflammation/cell proliferation (manifest for example, as alveolar Type II cell hyperplasia or fibrosis) will not incur any increased risk of lung cancer.

The Doll and Peto (1985) risk assessment for chrysotile-induced lung cancer was based on a linear no-threshold model applied to mortality data from chrysotile textile manufacture. However, the balance of toxicological evidence does not support the no-threshold model for asbestos-induced lung cancer. A practical threshold is likely.

Very few cases of mesothelioma can be reliably attributed to chrysotile despite the many thousands of workers who have had massive and prolonged exposures to this type of asbestos. In contrast, mesotheliomas have been observed among some workers who experienced only brief exposures to amphiboles. These differences are most likely explained by the limited durability of chrysotile in the lungs, in contrast to the amphiboles which are more persistent. It would appear that for a fixed level of exposure, the risk of developing mesothelioma is much greater for amphiboles than for chrysotile.

Evidence from human studies suggests that amphibole asbestos may lead to the development of mesothelioma at lower levels of cumulative exposure than would be required to cause lung cancer. However, no reliable exposure-response curve can be constructed for asbestos-induced mesothelioma either in animals or in humans, and although a threshold could be postulated on theoretical grounds, the available data do not allow the identification of a threshold level of exposure below which there would be no risk.

Deposition and clearance

The regional deposition efficiency of inhaled fibres is largely a function of fibre diameter and density, with length and aspect ratio being of minor importance. Maximum alveolar deposition efficiency of inhaled fibres in humans is expected to occur with mineral fibre diameters of about 1 μm , and to fall off with values above this,

so that above 3 μm in diameter, fibres would be essentially non-respirable. For organic fibres with a lower density, values slightly greater than this may apply.

Clearance of fibres which deposit in the alveolar regions of the lung takes place by macrophage-mediated phagocytosis which is highly effective for short fibres ($<5 \mu\text{m}$), but becomes increasingly difficult with increasing fibre length, so that clearance by this mechanism will be negligible for fibres of lengths around 16 μm or more. In the longer term, some fibres, depending on chemical composition, will undergo dissolution or leaching of particular elements from the fibre structure. This may lead to fragmentation into shorter lengths which will facilitate clearance. The pulmonary clearance of chrysotile is more rapid than for amphibole fibres of similar dimensions.

Animal studies

Different routes of administration have been used in animal studies with fibres. Intrapleural (IPL) and intraperitoneal (IP) administrations provide direct exposure of fibres to the target tissue (mesothelial membranes), and therefore represent maximum sensitivity as a test for the capacity to produce mesothelioma; however, these routes appear to be overly sensitive in relation to mesothelioma, in that fibres which perhaps because of effective lung clearance mechanisms do not pose a hazard by the inhalation route, can elicit mesothelioma when instilled directly as a large bolus dose into the pleural or peritoneal cavities. Hence positive results should be treated with caution. In contrast, negative results for mesothelioma induction by mineral fibres in a well conducted IP or IPL study would suggest an absence of carcinogenic hazard towards the mesothelial tissues. IPL and IP studies may be inappropriate for investigating organic fibres, because the physical properties of these fibres preclude the ability to inject a homogeneous suspension. In this case negative data may be unreliable.

Intratracheal (IT) instillation, whilst addressing the same route of administration as inhalation, does not mimic the pattern of pulmonary deposition for inhaled fibres. The same dose delivered as a single bolus may elicit a more severe inflammatory response in the lungs than when delivered gradually by inhalation. Therefore, for the purpose of evaluating potential human health effects of fibres, small doses given repeatedly, perhaps once or twice weekly over many months, should provide the most meaningful results.

It would seem sensible to regard positive carcinogenicity findings in an IT study as valid evidence of carcinogenic potential for inhaled fibres unless counteracted by negative results from one or more well conducted inhalation studies. In contrast, negative results from a well conducted IT study would suggest an absence of hazard, and in the context of a fibre toxicity testing strategy, a move to inhalation carcinogenicity testing would not be justified.

The inhalation route is of most relevance to human exposures to fibres. Therefore animal studies using this exposure route should provide a clearer basis for hazard identification and for investigating dose-response relationships. Inhalation studies in rats are able to demonstrate the known human health hazards of asbestos. However, in general, very few mesotheliomas can be produced with this exposure route, therefore large group sizes of rats (≥ 100) are needed to reliably identify this end-point.

Mechanisms of fibre pathogenicity

There has been considerable progress in recent years towards understanding the mechanisms of fibre toxicity and carcinogenicity. The development of pulmonary interstitial fibrosis is preceded by injury to the alveolar and bronchiolar epithelium. This results from direct toxicity caused by long fibres, but not short, and indirect toxicity caused by the release of oxidants and enzymes from macrophages and neutrophils following the incomplete phagocytosis of long fibres. Cytotoxicity may also result from

reactions catalysed at the fibre surface leading to the generation of oxygen-containing free radicals.

Airway epithelial damage may facilitate the passage of fibres from the airspaces into the pulmonary interstitium, which appears to be important in the development of pulmonary interstitial fibrosis. The mechanism for the development of pulmonary fibrosis is thought to result from the ability of fibres to provoke a chronic enhancement of the secretion of cytokine growth factor from effector cells.

There is evidence for an association between fibre-induced pulmonary interstitial fibrosis and lung cancer, consistent with the view that the enhanced rates of cell proliferation associated with chronic inflammation and fibrogenesis predispose to neoplastic cell transformation. There is a lack of convincing evidence for the ability of fibres to elicit any direct genetic changes which might also lead on to carcinogenesis.

The mechanisms of fibre induced mesothelioma are probably similar in principle to those for lung cancer, involving chronic inflammation and increased cell proliferation eventually leading to neoplastic transformation. However, there are still some uncertainties, for example, regarding the process and importance of fibre translocation to the mesothelial tissues.

Recent evidence shows that pleural changes can be elicited by indirect effects not involving any actual fibre penetration to the pleural membranes, but resulting from the deposition of fibres in the alveolar regions of the lung in close proximity to the sub-pleural membrane. The significance of this in relation to pleural toxicity is unclear.

Fibre toxicity testing strategy

The successful design of a fibre toxicity testing strategy requires an understanding of the mechanisms of fibre toxicity, and also of which physico-chemical properties most closely relate to toxicological hazard. There are still some uncertainties in these areas. Furthermore, the lack of standardised test methods has been a limiting factor. However, from the progress which has been made in the field of fibre toxicology in recent years, a testing strategy can be proposed which should provide a rational basis for hazard identification.

Although it is not possible, based on current knowledge, to make a precise prediction of toxicological hazard based on measures of physico-chemical properties, certain properties, such as fibre solubility and surface reactivity are thought to be determinants of toxicity. Therefore measures of these properties, when considered in conjunction with other test data, should provide useful indices of potential toxicity.

There is now sufficient understanding of the underlying mechanisms of fibre toxicity to allow meaningful studies of inflammatory, cytotoxic and proliferative effects in short-term animal and *in vitro* tests. The results from a limited number of such tests should enable decisions to be made on potential toxicity and needs for further testing. Fibres which proved to be relatively soluble, with low surface reactivity, and of low biological activity in well designed short-term toxicity tests, might be judged to be of low concern, and no further testing might be warranted. If these criteria are not met, then the next stage in a toxicity testing strategy would be to conduct a joint lung clearance and histopathology study of perhaps 6 months duration. The results from such a study should enable an evaluation of fibrogenic potential as well as provide evidence for fibre dissolution/ leaching and overall clearance kinetics. There should be sufficient information available at this stage in the testing strategy to allow at least cautious predictions of carcinogenic potential. Fibres which have proved to be cytotoxic, capable of inducing cell proliferation and fibrogenesis, and with limited evidence of lung clearance, might be regarded as potentially carcinogenic, and should be treated as such, unless counteracted by negative results in a well conducted life-time carcinogenicity study.

Relationship between fibre size and toxicity

The definition of a regulated fibres as used for counting purposes according to the UK Health & Safety Executive and the World Health Organisation is that of a particle of length $>5 \mu\text{m}$, and diameter $<3 \mu\text{m}$, and with an aspect ratio (length to diameter) of $>3:1$. There is good evidence that longer fibres are more toxic than equal masses of shorter fibres of the same composition. Evidence from animal studies suggests that short fibres ($<5 \mu\text{m}$) pose little if any concern for disease development at any site; fibres of lengths at least in the region of $10\text{--}15 \mu\text{m}$ are necessary to produce disease in the lungs; but shorter fibres in the region of $8\text{--}10 \mu\text{m}$ can cause mesothelioma. There is no biological reason to suppose that a sharp cut-off value in fibre length would separate hazardous from non-hazardous fibres. This suggests that there would be no justification for increasing the current value of $5 \mu\text{m}$ as the length of a 'regulated' fibre.

There is little evidence available on the role of fibre diameter. Finer fibres may appear to be more toxic simply due to their greater efficiency of lung deposition following inhalation exposure. There is no evidence that thinner fibres are more toxic than thicker fibres at a cellular level, when comparisons are based on numbers of fibres. Overall, based on considerations of patterns of regional deposition in the respiratory tract in relation to disease development, it is concluded that the focus of concern for counting purposes should continue to be with those fibres which are deemed to be respirable ie capable of depositing within the broncho-alveolar regions of the lung. For mineral fibres this would include all fibres $<3 \mu\text{m}$ in diameter. There are no toxicological reasons to suggest that the minimum diameter for a regulated fibres should be reduced below the current value. There is a lack of specific toxicological evidence relating to aspect ratio.

Section 1

PROPERTIES AND USES OF FIBRES

The following is a basic and brief guide to the main types and characteristics of fibres.

What are fibres?

1.1 The definition of a regulated fibre as used for counting purposes according to the WHO and the UK Health and Safety Executive is that of a particle of length $> 5 \mu\text{m}$, and diameter $< 3 \mu\text{m}$, and with an aspect ratio (length to diameter) of $> 3:1$ as measured by phase contrast optical microscopy (EH 10/90) using the membrane filter method. This definition applies to any particle fulfilling these criteria regardless of chemical composition or mineralogical features.

1.2 It should be noted that this definition applies to 'regulated fibres', and that fibres can exist outside of the specified size range. For example, short fibre chrysotile asbestos ($< 2 \mu\text{m}$ in length), is ubiquitous in air and water, can be found in the lungs of all sectors of the general population, but would not be counted when detected in air samples as a fibre for regulatory purposes. The toxicological justification for the definition of a regulated fibre is given in Section 7 of this document.

Naturally occurring mineral fibres

Asbestos

1.3 'Asbestos' is a commercial term which covers a number of naturally occurring silicate minerals whose crystals grow in the 'fibrous habit' (IPCS 1986). Asbestos minerals fall into two groups (serpentines and amphiboles) which can be differentiated on morphological and mineralogical grounds. Chrysotile (white asbestos), is the only member of the serpentine group, and crocidolite and amosite (blue and brown asbestos), are amphiboles. Other amphiboles are tremolite - found as a contaminant in chrysotile and talc deposits, and anthophyllite (mined in Finland).

1.4 Asbestos fibres exist in parallel bundles which tend to split longitudinally when subjected to pressure, forming finer and finer fibres which maintain a high aspect ratio. Chrysotile tends to split much more than the other forms of asbestos. Chrysotile fibres exist in the form of hollow scrolls, and may appear curved, whereas crocidolite and amosite fibres tend to be straight and splinter-like.

1.5 The asbestos minerals possess a number of useful properties including high tensile strength, good thermal and electrical insulating properties, and resistance to chemical attack. Asbestos also possesses an inherent flexibility and strength, enabling it to be woven into yarns and textiles. It is elastically compressible, hence its use in packings, jointings and seals. Asbestos can be used in friction materials (brake-linings), and is used to reinforce concrete, plastic and resins. The large surface area of asbestos fibres is responsible for its usefulness in filtration applications.

Other Naturally Occurring Mineral Fibres

Fibrous Zeolites

1.6 Zeolites are crystalline aluminosilicates (IPCS, 1986; Hodgson, 1993). More than 30 naturally occurring forms exist but among these only erionite has been of commercial relevance, as it was used in concrete aggregate and in road surfacing. The interest surrounding erionite lies in its outstanding pathogenicity, having led to large number of mesothelioma deaths in parts of Turkey where it is found in natural rock

outcrops, and in its unusually aggressive ability to cause mesothelioma on inhalation in laboratory rats.

Fibrous Clays

1.7 Attapulgite (also known as palygorskite) and sepiolite are fibrous clay minerals. Attapulgite is the main constituent of Fuller's Earth, and is used in cat litter. Both attapulgite and sepiolite are used in pharmaceuticals, as thickeners in paints, and as absorbants for oils, fats and waxes. Sepiolite is used in motor vehicle underbody sealants, and as a crosslinking agent between synthetic organic fibres and cement in fibre-cement systems.

Other Fibrous Silicates

1.8 Wollastonite is a simple calcium orthosilicate and has a high temperature resistance approaching 1500 C (Hodgson, 1993). Its main commercial use is in ceramic ware, fibre reinforced cements, friction products and thermal insulation materials.

Man-made mineral fibres

1.9 Man-made mineral fibres (MMMF) or man-made vitreous fibres (MMVF), are manufactured from glass, rock, slag or clay. MMMFs fall into a number of categories including: continuous filament glass fibres, insulation wool (including rock/slag wool and glass wool), and ceramic fibres. Most are vitreous (amorphous) silicate-based fibres, but the group does include some speciality fibres such as the crystalline silicon carbide 'whiskers'.

1.10 Glass fibres are manufactured using finely powdered sand as the main source of silicon, and kaolin clay and synthetic aluminium oxides are the main sources of aluminium. The refractory ceramic fibres (RCFs) are produced from melting kaolin clays or from oxides of aluminium or silicon.

1.11 The processes by which MMMFs are manufactured may be broadly divided into two types; one involves extrusion of the molten raw materials through nozzles (drawing process), which gives rise to continuous filament fibres. The diameter of such fibres is tightly controlled, so there is little deviation about the mean nominal diameter size. The continuous filament glass fibres are produced with diameters ranging from 3-25 µm, and so are largely non-respirable.

1.12 The insulation wools and also RCFs are typically manufactured by spinning or blowing processes. The mean diameter of most wools is about 4 µm, but there is a wide spread of diameter values about the mean, so that there will always be a significant respirable fraction. The finer fibres are those which are more likely to become airborne, and also to remain lofted (thicker heavier fibres will sediment out) so that the percentage of fine airborne fibres associated with a particular use or process will be greater than that in the bulk product. This distinction is important because it demonstrates that the descriptions of the nominal fibre diameters given for the bulk product do not reflect the fibre size distributions to which workers may be exposed.

Apart from these two broad types of manufacturing process there are other speciality processes involved, eg the production of the highly crystalline silicon carbide 'whiskers' involves deposition of the solid material from the vapour phase.

1.14 Unlike the naturally occurring asbestos fibres, the MMMFs do not consist of parallel bundles of fibres which are capable of splitting lengthways, giving rise to finer and finer fibres. Rather, MMMFs tend to break transversely giving rise to shorter fibres of the same diameter.

Synthetic organic fibres

Carbon/graphite fibres

1.15 Carbon/graphite fibres are produced by high temperature processing of rayon (regenerated cellulose), pitch (coal tar or petroleum residue) or polyacrylonitrile (IPCS, 1993). Reported diameters for carbon fibres range between 5-15 μm . Carbon fibres possess a number of useful properties including flexibility, high electrical and thermal conductivity, high strength, corrosion resistance, they are lightweight and refractile, and are chemically inert except to oxidation.

Polyamide (aramid) fibres

1.16 Aramid fibres are long-chain polymers formed from aromatic diamines and aromatic diacid chlorides (IPCS, 1993). They occur in two forms which differ in the substitution positions on the aromatic rings ie meta-aramids (Nomex[®]), and the para-aramids, marketed mainly as 'Kevlar[®]' and 'Twaron[®]'.

1.17 Para-aramids are manufactured as continuous yarns with individual filaments having nominal diameters of 12-15 μm . They have a highly oriented crystalline structure which can lead to the peeling from the main fibre surface of sub-fibres with diameters in the submicron range.

1.18 Aramid fibres possess a range of useful qualities including high tensile and dielectric strengths, and heat resistance. They are used as asbestos substitutes in friction formulations. Meta-aramids are not as strong as the para-aramids due to differences in chemical structure. Meta-aramid fibres are used in flame resistant garments. Para-aramid fibres are used in the manufacture of gloves for industrial and domestic use, high strength ropes, and in composites for a range of sporting products such as tennis rackets.

Polyolefin fibres

1.19 Polyolefin fibres are long-chain polymers whose monomeric substituents largely consist of ethylene, propylene, or other olefin units (IPCS, 1993). Most have nominal diameters which are outside the respirable range, except for the microfibrils which have diameters ranging from 0.1-2 μm . Polyolefin fibres are extremely hydrophobic, and are unreactive with a wide variety of inorganic acids and bases and organic solvents at room temperature. However, the use of these fibres is limited by their low melting points (116-179 C). The uses of polyolefin fibres include carpet backings, textiles, ropes, synthetic turf, and fishing lines.

Section 2

HUMAN HEALTH EFFECTS FROM EXPOSURE TO ASBESTOS

2.1 The aim of this section is to provide a summary of the human evidence relating to asbestos, because this may serve as a benchmark by which to evaluate the potential health effects of other fibrous materials. In 1985 Doll and Peto published a comprehensive review on asbestos for the Health and Safety Commission. Therefore, it is not the intention to provide another detailed overview of the epidemiological database on asbestos, but rather to focus on more recent views and evidence relating to the dose-response characteristics for main asbestos-related health effects.

2.2 The pathology and epidemiology of the asbestos-related diseases have been well described in the literature (Doll and Peto, 1985; IPCS, 1986; Craighead, 1982; Roggli, 1990; Selikoff, 1990; McDonald and McDonald, 1991; Liddell, 1991 and Hughes, 1991). Inhalation of asbestos can lead to pulmonary fibrosis (asbestosis); cancer of the lung (chiefly bronchial carcinoma); mesothelioma (chiefly of the pleura but also of the peritoneum); pleural disease (diffuse pleural thickening); and a benign condition of the pleura known as pleural plaques. It is clear from these reviews that while all forms of asbestos are implicated in these diseases, there are marked differences in the degree of hazard among the different asbestos fibre types.

Introduction

2.3 Widespread recognition of the health effects of asbestos has led to legal restrictions imposed on the use of asbestos, and consequently to a well established trend towards the development and use of asbestos substitutes, so that the industrial use of asbestos has declined substantially. For some forms of asbestos (crocidolite and amosite) the legal restrictions in force in the UK have led to the prohibition of their import, supply and use (Asbestos Prohibitions Regulations 1992). However, exposure to these forms of asbestos may still occur in stripping and removal of existing insulation, and in demolition work.

2.4 Standards of occupational hygiene have improved considerably, and the massive exposures to fibres typical of early industrial processes should no longer occur (in developed countries). However, despite growing awareness of the nature of the asbestos-related diseases, the world production and use of asbestos continued to increase significantly, increasing by 50% between 1964 and 1973, and did not start to level out until the 1980s.

2.5 Within the UK, the number of disablement benefits for asbestosis, and the death rates from lung cancer associated with asbestos and also for mesothelioma, continue to rise. This rising trend of morbidity and mortality associated with asbestos shows no sign of reaching a plateau. Over 1000 deaths were recorded in the UK in 1991 for mesothelioma, a 15% rise on the 1990 total (HSC Annual Report 1992/92). The estimated total number of deaths in the UK due to asbestos (mesothelioma, asbestosis and lung cancer) is now approaching 3000-3500 annually.

Historical overview

2.6 The history of large scale mining and industrial use of asbestos goes back to the late nineteenth century. Asbestosis was first identified as a definite pathological and clinical entity in the 1920s. Public awareness that lung tumours were causally related to asbestos did not fully emerge until the epidemiological study of chrysotile textile workers in Rochdale by Doll in 1955. The association between asbestos and mesothelioma was first demonstrated in a study in South African miners (Wagner *et al.*, 1960).

2.7 A number of factors are responsible for the delay in recognition of the asbestos-related diseases. Such factors include: the long time period (frequently 20 years or more) between first exposure and the onset of clinical symptoms; the fact that asbestosis can lead to cardiovascular sequelae which may have been subsequently recorded as the cause of death on the death certificate; also, mesothelioma is extremely rare and therefore may have been under-diagnosed in the early days due to lack of familiarity with this condition among physicians.

Lung fibre burden studies

2.8 A major limitation to defining the dose-response relationships for the asbestos-related diseases is the lack of accurate exposure data. Examination of the human lung fibre content can potentially offer a valuable source of information on past exposure, although such data need to be interpreted with care. Lung fibre analyses are usually obtained following digestion of a small sample of lung tissue in bleach or potassium hydroxide, or by ashing the lung tissue, followed by collection of the extracted fibres onto a filter for counting either by phase contrast optical microscopy (PCOM) or electron microscopy methods (Churg, 1993). PCOM will miss fibres finer than about 0.25 μm in diameter.

2.9 The limitations and difficulties in lung fibre burden analysis are highlighted in recent reviews by Case (1994) and Churg (1993). Interlaboratory variation can account for large differences (orders of magnitude) in lung fibre content values (Roggli, 1990). Even within the same individual, there is extensive variation in fibre concentrations from lobe to lobe, from site to site within lobes, and even from immediately adjacent samples.

2.10 A further difficulty is the lack of standardised criteria for the counting of fibres in lung tissue samples. Some investigators count all asbestos particles with an aspect ratio of $>3:1$, even down to a length of 1 μm or less, and do not report on the fibre size distribution. Other investigators count only those fibres which fulfill the criteria for a 'regulated fibre' used in airborne monitoring (see para 7.1), and thus count only fibres with a length of at least 5 μm . The latter would seem to be more appropriate in view of the evidence for the importance of fibre length in toxicity (see Section 7). Due to the ubiquitous presence of short fibre ($<5 \mu\text{m}$) chrysotile in air and water, contamination of laboratory reagents and fixatives is common. Solutions should be prepared by filtration in a laminar flow clean room. The finding of short fibre chrysotile in lung samples must always be regarded as suspect if contamination has not been adequately controlled.

2.11 Studies have shown that asbestos fibres can be found in the lungs of all population groups, even those with no known occupational exposure to asbestos, reflecting widespread contamination of the urban air. The importance of properly selected control groups in considering an aetiological role for asbestos in disease development based on lung fibre analyses is discussed by Case (1994).

2.12 Analysis of lung fibre burden data in human cases is usually only performed at one point in time (autopsy), which cannot adequately take account of the dynamic nature of the lung fibre burden, particularly for chrysotile, which is believed to undergo more effective lung clearance than amphibole forms of asbestos. Accurate knowledge of the occupational history is important to assist in the interpretation of lung fibre data; such knowledge is needed for estimations of the times since first and last exposure and the likely residence time of fibres in the lungs.

2.13 Despite the precautions which must be observed in the analysis of human lung fibre content, well designed studies may become increasingly useful in assessment of past exposure. Overall, the available studies suggest that the production of asbestosis or lung cancer requires a sufficient pulmonary burden of fibres to be accumulated and maintained. Results obtained from examinations of the lungs of asbestos workers at

autopsy suggest that asbestosis will not occur until the lung fibre burden exceeds 5×10^5 fibres/gram of dried lung (fibres $>5 \mu\text{m}$ in length). In severe cases of asbestosis, fibre counts can be as high as 1×10^{10} /gram of dried lung. These observations are consistent with epidemiological data which indicate that asbestosis occurs in individuals with prolonged and heavy exposure to asbestos. In cases of carcinoma, lung fibre burdens occur in much the same range as lung burdens for asbestosis (Davis and Donaldson, 1993).

2.14 There is little published work on the fibre content of the pleural tissues. When asbestos fibres are found in the pleura, it is claimed that mainly short fibre ($<2 \mu\text{m}$) chrysotile is most often found (Sebastien, 1991). The fibre burden in the pulmonary parenchyma in cases of mesothelioma has been found to range from the highest burdens associated with asbestosis, to the background level for the rural population (Roggli, 1990). Human evidence also indicates that mesothelioma can occur in the absence of asbestosis, and therefore can occur at lower fibre burdens than are needed to induce fibrosis and lung cancer.

Dose-response relationships for the asbestos-related diseases

Asbestosis- pulmonary interstitial fibrosis

2.15 Asbestosis is diffuse interstitial pulmonary fibrosis resulting from the accumulation of airborne asbestos in the lungs (Craighead, 1982). The development of asbestosis commences with a thickening of the walls of the respiratory bronchioles and alveolar ducts due to the accumulation of reticulin and collagen, often with the replacement of flattened epithelial cells with cuboidal cells. As the condition progresses the alveolar septa adjacent to terminal bronchioles become thickened and fibrosed until large areas of the parenchyma become involved. Post-mortem findings have shown that in advanced cases of asbestosis the lung parenchyma takes on a honey-comb appearance that is most prominent in the lower lobes and sub-pleurally.

2.16 The development of asbestos-induced pulmonary fibrosis has been shown to follow a dose-response relationship in that increasing levels of exposure lead to higher incidences of the disease (Weill, 1994). However there are difficulties in defining this relationship both from the point of view of clinical diagnosis and from the generally limited exposure data, as described by Doll and Peto (1985). Because asbestosis is not always a fatal disease, mortality studies do not provide reliable information on the true rates of this disease, and thus less quantitative dose-response data are available than might be expected.

2.17 It has been argued that there may be sub-clinical changes induced by chrysotile (Doll and Peto, 1985; Hillerdal, 1994a), but it is generally agreed that low levels of exposure, even if they can initiate a fibrotic process in the lungs, will not likely progress to the point of clinical manifestation.

2.18 In a prospective study in New Orleans asbestos cement workers, very little radiological evidence of asbestosis was seen at cumulative exposures below 30-40 fibre.years/ml (Hughes and Weill, 1991). The evidence from the New Orleans cohort, in which crocidolite exposure occurred in one of the two plants studied, indicated that the prevalence and progression of asbestosis was greater with crocidolite than with chrysotile when total fibre exposure had been taken into account (Weill, 1994).

2.19 The fact that there have been no clinical cases of asbestosis among the general public also suggests that there must be a threshold level of exposure to asbestos which must be exceeded for this disease to occur. This conclusion is reinforced by the failure to detect any cases in over 600 members of the families of asbestos workers in the US (Doll and Peto, 1985).

2.20 Overall therefore, it appears that there will be a threshold level of exposure, at or below which no radiological or clinical manifestations of pulmonary fibrosis will be detectable.

2.21 However, both fibre type and the airborne fibre size distribution can be expected to influence the slope of the dose-response relationship. Therefore threshold values for clinically detectable asbestosis can be expected to vary accordingly. To illustrate this point, prevalence rates for radiologic asbestosis were of the order of 20% for cumulative exposure of approximately 25 fibre.years/ml in a Quebec textile plant using Quebec chrysotile, and only about 8% for a cumulative exposure of about 60 fibre.years/ml in Quebec miners and millers (Becklake, 1993). These differences may be attributable to differences in the fibre length distributions of the chrysotile in these occupational settings (see para 2.28).

2.22 The importance of resolving the issue of whether or not asbestosis is a threshold phenomenon stems from the putative relationship between asbestosis and lung cancer. This is discussed further below.

Lung carcinoma

2.23 All types of asbestos are implicated in the development of lung cancer. The lung cancers that are caused by asbestos are described as clinically indistinguishable from those caused by cigarette smoking (Doll and Peto, 1985). They occur principally in the main bronchi, but they also occur in the smaller bronchi and in the periphery of the lung and occur in a variety of histological forms.

2.24 Most exposures in the occupational setting (other than in mining and milling) have involved mixtures of asbestos fibre types, and therefore the human evidence does not allow a precise quantitative comparison of the risks from exposure to chrysotile and amphiboles. However, as concluded by a WHO task group, epidemiological evidence suggests that at comparable exposures, exposure to crocidolite poses a greater risk of lung cancer than chrysotile (IPCS, 1986).

Chrysotile-induced lung cancer

2.25 Lung cancer mortality has been investigated in cohort studies of workers exposed to chrysotile in mining and milling, and in textile, asbestos-cement and asbestos-friction product manufacture. Marked differences in the slopes of the exposure-response curves have been observed across these industries, with the steepest slopes being found in textile manufacture. In asbestos friction product manufacture, no significant increases in lung cancer mortality have been reported (Berry, 1994). In asbestos cement manufacture, although chrysotile has been the predominant form of asbestos used, most factories have used varying amounts of amphiboles (Weill, 1994). Where exposure was judged to be exclusively to chrysotile no excess lung cancer mortality has been noted.

2.26 The most comprehensive source of information on lung cancer mortality in chrysotile mining and milling comes from a study of a cohort of 11 000 Quebec chrysotile miners and millers born between 1881-1920, which has been followed up until 1988 (McDonald, 1993). The most recent update on this cohort includes an analysis by the subject-years method of the mortality between 1976-1988. These results show a sharp increase in standard mortality ratio (SMR) for lung cancer with cumulative exposures above 300 mpcf.y (millions of particles per cubic foot.years) but for seven categories of exposure up to this level the SMR fluctuated around 1.27 with no indication of trend. It was suggested by Liddell (1994) that the lung cancer SMR of 1.27 arose from comparatively heavy cigarette smoking. Converting from mpcf to f/ml is highly problematic with at least one order of magnitude in error to be expected, but on 'average' 3.5 f/ml was said to correspond to 1 mpcf. Using this conversion, the results suggest that no detectable increases in lung cancer would be anticipated with 20-year

exposures below 50 f/ml (8-hour TWA). Furthermore, the results from the update to 1988 were said to confirm and provide strong reinforcement of earlier findings from the same cohort.

2.27 These results are in marked contrast to those found in studies of chrysotile textile workers which are consistent in demonstrating steep increases in the risks of lung cancer and asbestosis with increasing cumulative exposure (Peto *et al*, 1985; McDonald *et al*, 1983a; McDonald *et al*, 1983b and Dement and Brown, 1994).

2.28 The reasons for the differences in lung cancer risks in textile workers and miners and millers are not entirely clear, but may be partly attributable to differences in the airborne fibre size distributions in these industries, and also to inaccuracies in the reported exposure estimates. As noted by Doll and Peto (1985), asbestos only constitutes about 20% of the ore that is mined, and it is progressively refined during processing. Chrysotile grades containing the longest silkiest fibres were reserved for distribution to textile manufacture. Thus it can be reasonably concluded that a higher proportion of shorter fibres and non-fibrous dust would have been present in the air in mining and milling than in textile manufacture. Dement (1991) found a higher proportion of longer ($>15 \mu\text{m}$) airborne fibres in textile manufacture compared with other chrysotile user industries.

2.29 In view of the marked differences in the risks of lung cancer observed in chrysotile mining and milling compared with textile manufacture, together with uncertainties in the reliability of the exposure estimates from these studies, the use of risk estimates derived from mining and milling are considered to be inappropriate for predicting risks in the user industries.

2.30 The data from a study of UK asbestos textile workers were used to develop a quantitative risk assessment for chrysotile-induced lung cancer (Doll and Peto, 1985). The study included an analysis of the mortality up to 1983 of a principal cohort of 3211 men employed between 1933 and 1974 (Peto *et al*, 1985). In this cohort, excess mortality from lung cancer was found to be concentrated in men with greater than 10 years service in the period 20 years or more after first employment (SMR = 209, against an SMR of 113 for all other men and at all other times). The analysis did not take account of smoking status.

2.31 Indices of cumulative exposure were calculated for each worker and were used to calculate dose-specific risk estimates based on the assumption of a linear no-threshold model. The estimates were broadly consistent with those obtained in a US study of chrysotile textile workers (McDonald *et al*, 1983a). Doll and Peto (1985) concluded that the central estimate of the life-long risk caused by 20 to 30 years exposure in chrysotile textile manufacture would be about 0.5% for exposures of 0.5 fibre/ml.

2.32 However, as noted by Peto *et al* (1985), the accuracy of this prediction is questionable, mainly due to limitations in the exposure data. Routine airborne monitoring only began in the UK textile plant in 1951, and was undertaken by means of static area sampling at selected sites. Until 1961 exposure measurements were of particle counts and many particles would not have been asbestos fibres, leading to uncertainties about the reliability of the conversion factor for particle to fibre counts. No measurements were available for what were considered to have been areas with the highest exposures.

2.33 Furthermore, the validity of the linear no-threshold model used to underpin the risk estimates for chrysotile-induced lung cancer may also be open to question. Due to the relatively high background rate of a disease such as lung cancer in the human population, it could be argued that epidemiological data alone are not able to clearly distinguish between the possibility of a threshold or a non-threshold model. Therefore it is important to consider other strands of evidence which may be relevant to understanding the nature of this dose-response relationship.

2.34 Although the dose-response relationship for asbestos-induced lung cancer has not been adequately investigated in animal studies, the results of well conducted inhalation carcinogenicity studies in rats with refractory ceramic fibres (RCFs) are consistent with a threshold model (see para 4.37). There is no reason to consider that there would be any significant differences in mechanisms of pulmonary toxicity between the asbestos group of mineral fibres and the RCFs (see para 5.21-5.23). Overall, the balance of toxicological evidence suggests that the linear no-threshold model for chrysotile-induced lung cancer may not be appropriate.

Evidence for an association between asbestosis and lung cancer

2.35 For many years human evidence has pointed towards an association between asbestosis and the risk of lung cancer. Both diseases show similar dose-response relationships with respect to asbestos exposure, show similar latent periods for development, and show a similar dependence on fibre length and composition. Lung fibre analyses indicate that both diseases require the accumulation of high concentrations of fibres (at least 10^5 fibres/dried gram of lung, see para 2.13). However, the question of whether asbestos-induced lung cancer can develop in the absence of asbestosis has been the subject of intense debate, fuelled perhaps by uncertainties regarding the mechanisms of development of these disease, and it has been argued that there is not necessarily a causal or precursor relationship between asbestosis and lung cancer (Abraham, 1994; Roggli *et al*, 1994).

2.36 Studies of workers known to be occupationally exposed to asbestos have consistently demonstrated, usually upon autopsy investigation, that asbestos-induced lung cancer is seen in association with pulmonary fibrosis (Doll, 1955 - chrysotile textile workers; Newhouse *et al*, 1985; Wagner *et al*, 1988 - asbestos factory workers; Sluis-Cremer and Beuzidenhout, 1989 - amphibole miners; Kipen *et al*, 1987 - asbestos insulation workers).

2.37 One occupational study involved a prospective design in which workers with and without radiologic evidence of pulmonary opacities were followed forward in time to investigate subsequent lung cancer mortality (Hughes and Weill, 1991). This was a study of 839 New Orleans asbestos cement workers in which excess lung cancer mortality was only seen in those who had radiological evidence of pre-existing asbestosis (profusion of small opacities of at least 1/0 in the International Labour Organisation classification) at the start of the mortality follow up in the 1960's. Among these workers there were 9 lung cancer deaths versus 2.1 expected. No excess of lung cancer was seen in those workers without pulmonary opacities, even in those with over 20 years of employment.

2.38 In contrast, a number of general population studies have been reported which suggest that lung cancer may be caused by asbestos in the absence of asbestosis (Wilkinson *et al*, 1995; Antilla *et al*, 1993; Karjalainen *et al*, 1994; and Hillerdal, 1994b). There are a number limitations with these studies, including: the lack of specific evidence relating to the nature and extent of past exposure to asbestos; the lack of a clear dose-response relationship between the inferred duration of exposure and risk of lung cancer; problems with the diagnosis of pulmonary fibrosis - in the Wilkinson study it was based on radiologic examination which is recognised to be lacking in sensitivity and may miss 20% of cases which can be detected histologically (Kipen *et al*, 1987), but also in this study one half of the lung field was blocked out so that the ability of observers to classify the X-ray films would have been restricted; or the authors failed to define the criteria for establishing pulmonary fibrosis when histological examination was involved; no adjustments were made for potential confounding due to other occupational exposures eg in foundry work and chromium plating. Due to the various limitations in these studies no firm conclusions can be drawn regarding a causal role for asbestos in the aetiology of the lung cancers among the subjects concerned.

2.39 Overall, it is considered that more weight should be attached to the findings of the occupational studies, most of which involved well defined cohorts of workers for whom considerable information was available on the nature and extent of past exposure to asbestos. These studies provide consistent support for an association between lung cancer and asbestosis. This association has also been noted in animal studies (see paras 4.31-4.33). Animal studies are particularly useful in this context because they offer the opportunity to examine the time course of disease development following exposure to fibres under well controlled conditions. Animal studies clearly demonstrate that fibre-induced lung cancer is preceded by the development of pulmonary fibrosis (see paras 4.31-4.40). They also demonstrate that exposures which are insufficient to cause pulmonary fibrosis do not lead to an increase in lung tumour incidence.

2.40 As discussed in para 5.26, various lines of experimental evidence indicate that pulmonary fibrosis and lung cancer both emanate from the same underlying chronic inflammatory condition. These observations suggest that exposures which are insufficient to elicit chronic inflammation/cell proliferation (manifest, for example, as alveolar Type II cell hyperplasia or fibrosis) will not incur any increased risk of lung cancer.

Mesothelioma

2.41 Malignant mesotheliomas of the pleura and peritoneum are rare in the general population. Pleural mesotheliomas originate from the serosal lining of the pleural cavity that surrounds the lungs. In the early stages of development the parietal pleura (the layer adjacent to the chest cavity) becomes thickened, followed by thickening of the visceral pleura (layer covering the lungs). In its fully developed form the tumour tends to surround one and sometimes both lungs and mediastinal structures (Craighead, 1982; Palekar, 1988). Peritoneal mesotheliomas surround the bowel and sometimes lead to obstruction. Once diagnosed, the prognosis for mesothelioma is extremely poor, and death usually occurs within 18 months.

2.42 There is sufficient evidence to indicate that for a given level of exposure, the risk of developing mesothelioma is far greater with amphibole forms of asbestos (particularly crocidolite and amosite) than with chrysotile. For example, out of about 1200 deaths which occurred in a cohort of Australian miners and millers of crocidolite, there were 94 cases of mesothelioma (Berry, 1991). The average duration of employment in these mines and mills was relatively brief (3-4 months), and almost 80% of the cohort were still alive and less than 65 years old at the time of the latest mortality update (suggesting that the eventual cumulative mortality from mesothelioma in this cohort will be substantially higher). In contrast, there were only 33 deaths from mesothelioma in the Quebec miners and millers of chrysotile out of a total of over 7000 deaths by 1988, at which time most of the cohort subjects had died or were lost to view (McDonald, 1993).

2.43 It has been suggested that the mesotheliomas seen in the chrysotile miners and millers may be attributable to the presence of very low levels of contamination of the ore with tremolite. This suggestion is supported by autopsy findings showing that tremolite concentrations exceed those of chrysotile in the lungs of chrysotile miners and millers, despite the fact that tremolite is only present as a minor contaminant in the ore (Sebastien *et al*, 1989). The matter is not entirely resolved. Overall, very few cases of mesothelioma can be reliably attributed to chrysotile, despite the large numbers of workers who have been heavily exposed (McDonald and McDonald, 1991).

2.44 There are uncertainties in defining the dose-response relationship for mesothelioma induced by asbestos, largely due to the lack of reliable quantitative exposure data in relation to this toxicological end-point. However, as with other occupationally related cancers, time since first exposure appears to be an important factor. Observations on the incidence of mesothelioma in North American insulation

workers suggested that the incidence of the disease increases in proportion to a power function of the time elapsed since first exposure, being less strongly influenced by the duration of exposure. Thus the risk assessment model produced by Doll and Peto (1985) was based on the assumption that risk is related approximately to the cube of the time since the exposure occurred (cubic residence time model).

2.45 Since publication of the Doll and Peto (1985) review, studies on amphibole miners and millers have been reported which provide some exposure data (Sluis Cremer *et al*, 1992 and Armstrong *et al*, 1988). In one of these studies, estimates of cumulative exposure were derived for each worker (Armstrong *et al*, 1988). Deaths from mesothelioma were seen even in workers with the lowest categories of cumulative exposure (<10 fibre.years/ml). However, these exposure values must be viewed with caution. Exposure monitoring was conducted only infrequently in these studies, and a variety of different dust measurement methods were used, such that it is not clear how well the reported values would equate to those obtained with modern methods.

2.46 Animal evidence is uninformative regarding the dose-response relationship for asbestos-induced mesothelioma. Too few mesotheliomas have occurred in animal inhalation studies to allow any meaningful conclusions to be drawn regarding the dose-response characteristics for this end-point (see Table 3 in Section 4). There is some evidence from epidemiological studies that mesothelioma may result from exposures which would be insufficient to cause lung cancer; in a study of mortality among UK dockyard workers exposed to a mixture of types of asbestos including amphiboles, there were 33 deaths from mesothelioma, but there were 11 fewer deaths from lung cancer than expected (Rossiter and Coles, 1980). Similarly, although there were 12 confirmed deaths from mesothelioma among workers in a UK factory making asbestos friction products, there were only 88 deaths from lung cancer, which was 19 fewer than expected based on national cause specific mortality rates (Berry, 1994). Most if not all of the mesothelioma deaths among the workers in this factory could be traced to the use of crocidolite which had been used in a special contract.

2.47 Overall, although theoretical considerations might suggest the possibility of a threshold for asbestos-induced mesothelioma, it is not possible from the available human and animal data to identify a threshold level of exposure at or below which there would be no increased risk. Furthermore, human evidence indicates that amphibole asbestos may lead to the development of mesothelioma at lower levels of cumulative exposure than would be required to cause lung cancer. It is also clear from human studies that the risk of mesothelioma is substantially greater with amphiboles than with chrysotile at equivalent levels of exposure. This difference is most likely explained by the far greater persistence and durability of amphiboles in the lungs compared with chrysotile.

Asbestos control limits

2.48 There are specific regulations in force in the UK governing the use and handling of asbestos (Control of Asbestos at Work Regulations, 1987). The Control Limits which apply in the UK to occupational exposures to asbestos correspond in principle to MELs, and thus do not represent health-based limits.

Table 1 Asbestos Control Limits * (not reproduced in full)

	Fibres per millilitre of air averaged over	
	4 hours	10 minutes
Chrysotile	0.5	1.5
Crocidolite/Amosite	0.2	0.6

*These limits apply to fibres having a length of at least 5 μm and a diameter of 3 μm or less, with an aspect ratio of 3:1 or more.

Summary of human health effects from exposure to asbestos

2.49 All forms of asbestos may cause pulmonary fibrosis, lung cancer and mesothelioma, but the degree of hazard depends on fibre type (greater with amphiboles than with chrysotile) and on the fibre size (long fibres more hazardous than short). From this it is apparent that meaningful comparisons of the risks observed in different occupational cohorts should take careful account of differences not just in fibre type, but also in the airborne fibre size distributions.

2.50 Although even low levels of asbestos may be able to trigger sub-clinical fibrotic pulmonary reactions, it is generally agreed that there will be a threshold level of exposure below which no radiological or clinical manifestation of asbestosis will occur. The value for the threshold, and indeed the slope of the dose-response curve, depends on the fibre type, and on the fibre size distribution in the workplace.

2.51 There appears to be an association between pulmonary fibrosis and lung cancer in that both diseases show a similar dose-response relationship with respect to asbestos exposure, show similar latent periods for development, show a similar dependence on fibre type and size, and both diseases emanate from the same underlying chronic inflammatory condition. These observations suggest that asbestos-induced lung cancer, like fibrosis, is a threshold phenomenon. It can be concluded that exposures which are insufficient to elicit chronic inflammation/cell proliferation (manifest for example, as alveolar Type II cell hyperplasia or fibrosis) will not incur any increased risk of lung cancer.

2.52 The Doll and Peto (1985) risk assessment for chrysotile-induced lung cancer was based on a linear no-threshold model applied to lung cancer mortality data from chrysotile textile manufacture. However, the balance of toxicological evidence does not support the no-threshold model for asbestos-induced lung cancer. A practical threshold is likely.

2.53 Very few cases of mesothelioma can be reliably attributed to chrysotile despite the many thousands of workers who have had massive and prolonged exposures to this type of asbestos. In contrast, substantial numbers of mesothelioma have been observed in workers exposed to amphibole forms of asbestos, even among some workers who experienced only brief exposures. These differences are most likely explained by the limited durability of chrysotile in the lungs, in contrast to the amphiboles which are more persistent. It would appear that for a fixed level of exposure, the risk of developing mesothelioma is very much greater for amphiboles than for chrysotile.

2.54 Evidence from human studies suggests that amphibole asbestos may lead to the development of mesothelioma at lower levels of cumulative exposure than would be required to cause lung cancer. However, no reliable exposure-response curve can be constructed for asbestos-induced mesothelioma either in animals or in humans, and although a threshold could be postulated on theoretical grounds, the available data do not allow the identification of a threshold level of exposure below which there would be no risk.

Section 3

DEPOSITION AND CLEARANCE

3.1 The deposition and clearance of fibres has been reviewed extensively including IPCS (1988), Lippmann (1990), Sebastien (1991) and Jones (1993). Although there is some information in the literature concerning human lung fibre burdens, the majority of information relevant to the subject of deposition and clearance of fibres comes from animal studies, chiefly in the rat.

Deposition

3.2 The regional deposition efficiency of inhaled particles is a function of aerodynamic diameter. For fibres, aerodynamic diameter depends on actual cross-sectional diameter and also on density, with fibre length and aspect ratio being of minor importance (Jones, 1993). It is estimated that the aerodynamic diameter of fibres is approximately equivalent to three times the value of the fibre diameter, so that for example, a mineral fibre with a diameter of 1 μm would have an aerodynamic diameter of 3 μm (Timbrell, 1982). Based on what is known about the regional deposition efficiency of spherical particles in the human lung, it can be concluded that maximum alveolar deposition efficiency for inhaled mineral fibres will occur with fibre diameters of about 1 μm , and will decrease with values greater than this, so that fibres with actual diameters of $>3 \mu\text{m}$ would be essentially non-respirable in man (Walton, 1982). A somewhat lower corresponding value of about 2 μm applies to rats (Morgan, 1980). For organic fibres with a lower density, it has been suggested that fibres of slightly larger diameters would be respirable than is the case for mineral fibres (Brown, 1990).

3.3 Interception is an important mechanism of deposition for fibres, and increases with increasing fibre length. Interception occurs when although the main body of the fibre continues to move along with the airstream, an edge of the fibre touches an epithelial surface. Fibres are deposited by interception mainly in the nasal passages and respiratory bronchioles. However, fibres with lengths $>200 \mu\text{m}$ have been found in the alveolar regions of the human lung, indicating that even for very long fibres, not all are intercepted (Craighead, 1982).

Species differences

3.4 Although aerodynamic diameter is important in determining the site and extent of deposition in the respiratory tract, the diameter and branching pattern of the airways are also significant factors. The rat is often used in fibre toxicity studies, and yet there are differences in the morphology of the rat and human respiratory systems. In the rat the branching pattern of the airway passages is monopodal (main airway continues unchanged in direction and minor branches emerge at intervals). In the human the airway branching pattern is bipodal (major airway divides into two similar daughter passages coming off at equal angles). An analysis of the differences in the morphometry and flow patterns in the the respiratory tracts of the rat and the human was carried out by Jones (1993). It was concluded that the differences between the rat and the human have counterbalancing effects, so that pattern of regional deposition of long fibres into the lung is likely to be broadly similar in both species.

Experimental observations in animals

3.5 The pulmonary deposition of inhaled chrysotile in rats has been studied by Brody *et al* (1981) using electron microscopy techniques. They found that shortly after a brief inhalation exposure, fibres were deposited preferentially around the junctions of the terminal bronchioles and alveolar ducts, particularly on early alveolar duct bifurcations. These are the sites of initial development of asbestosis (Craighead *et al*, 1982). Inhalation studies with crocidolite reviewed by

Rom *et al* (1991), with silicon carbide whiskers (Lapin *et al* 1991), and with aramid fibres (Lee *et al*, 1988) have shown similar deposition patterns to those observed with chrysotile.

3.6 In a study reported by Morgan *et al* (1977) rats were exposed nose-only for 30 minutes to aerosols of radioactive samples of different types of asbestos. Autoradiographs of lung sections of rats killed immediately post-exposure indicated that alveolar deposition was relatively uniform, and occurred right up to the periphery of the lung. 'Hot-spots' on autoradiographs due to accumulation of fibres in the smaller conducting airways, particularly at bifurcations, were noted. However, over a period of several months the pattern of distribution was found to alter, and fibres were seen to accumulate in sub-pleural foci, a phenomenon referred to as 'pleural drift'. Coin *et al* (1992) showed that there was no difference in the extent of pulmonary deposition of chrysotile between central and peripheral regions of the lung in rats for up to 29 days following a 3-hour exposure. These results may be considered somewhat surprising since one might expect greater initial deposition in central compared with peripheral regions. However, histologic analysis revealed that the first alveolar duct bifurcations can occur within 0.5 mm of the visceral pleura, and hence this expectation would not be valid.

3.7 Asbestos fibres (crocidolite, amosite and chrysotile) have all been observed to undergo longitudinal splitting in the lung, a process known as fibrillation (Coffin *et al*, 1992). This is particularly prominent with chrysotile. Thus with time, the actual number of asbestos fibres present in the lungs after a fixed exposure may increase.

Lung deposition and retention data

3.8 Few quantitative data are available on the pulmonary deposition and retention of inhaled fibres. Morgan *et al* (1980) exposed rats nose-only for 2-3 hours to aerosols of sized glass fibres which had been irradiated by neutron bombardment. The fibres had nominal diameters of 1.5 or 3 μm , and lengths ranging from 5 to 60 μm . Animals were killed either immediately or at 2 days post-exposure for radiometric measurements of regional fibre deposition in the respiratory and GI tract, pelt and faeces. Alveolar deposition was found to decrease with increasing fibre length due to interception of the longer fibres in the upper airways. All of the inhaled longer fibres (>30 μm) were deposited, mainly in the upper respiratory tract. Alveolar deposition of these longer fibres, and those of 3 μm diameter, was said to be negligible. The results showed that in the rat, alveolar deposition reached peak values (~12% of inhaled dose) for fibres of aerodynamic diameter of about 2 μm (presumably actual diameter ~0.7 μm).

3.9 Hammad *et al* (1982) studied the lung retention of MMMFs in rats, sacrificed 5 days after the end of a 6-day period of inhalation exposure to fibres with count median diameter (CMD) 1.1-1.3 μm , and count median length (CML) 8-20 μm . They found increased alveolar retention of fibres with decreasing diameter. Percentage retention was in terms of fibre number rather than mass. Maximum retention was 7.6% for fibres <0.5 μm in diameter and 21 μm in length. For fibres of 40 μm in length and <0.5 μm diameter, retention was about 3%.

Clearance

3.10 In general, the potential for fibre-induced chronic effects such as pulmonary fibrosis or lung tumours is less likely to occur with fibre-types or fibre dimensions that permit rapid clearance from the lung.

3.11 The clearance of inhaled fibres from the lungs may be broadly divided into two types of processes, ie mechanical and chemical. The mechanical processes encompass those responsible for the migration or translocation of fibres away from the initial sites

of alveolar and airway deposition to other sites, for example to the more proximal airways or to regional lymph nodes. Chemically mediated clearance involves the dissolution of fibres within the lung tissues, and is a function mainly of chemical composition.

Mechanical clearance- mechanisms

3.12 Mechanical clearance depends initially on the site of deposition ie in the ciliated or non-ciliated airway passages. Fibres which are deposited on the ciliated epithelial surfaces of the tracheobronchial passages are transported to the pharynx via the 'mucociliary escalator' and are then swallowed. The beating action of the cilia is rapid and efficient so that clearance of particles deposited in this region of the respiratory tract is anticipated to be effectively complete within 24 hours. Berke and Roslinski (1971) found that mucociliary clearance rates in the trachea of the rat were of the order of several mm per minute.

3.13 For fibres which deposit in the non-ciliated airway passages, beyond the terminal bronchioles in the alveolar region of the lung, clearance is mediated by the phagocytic actions of alveolar macrophages (AMs). The particle-laden AMs move by amoeboid action towards the bronchiolar lumen, and will be subsequently transported along the mucociliary escalator. However, clearance by this mechanism is only effective for fibres which are short enough to be fully engulfed by AMs. Long fibres (~20 µm) may be partly engulfed by several macrophages simultaneously, preventing the effective removal of such fibres from the lung, and possibly initiating a granulomatous response.

Experimental data on lung clearance of fibres

3.14 Bellman *et al* (1987) measured the pulmonary clearance of some MMMF, crocidolite and chrysotile in the rat following intratracheal (IT) instillation in a 2-year study. Parameters measured included the numbers and dimensions of fibres recovered from the lung ash (grouped into fibres >5 µm and <5 µm in length), and the leaching of various elements from the fibres was also studied. The study was able to demonstrate the importance of macrophage clearance mechanisms for short fibres, and provide evidence for fibre dissolution over the long term.

3.15 No change in fibre number of fibres >5 µm in length was found for crocidolite over 2-years (estimated half-time for clearance of 1000 days). For crocidolite fibres shorter than this some lung clearance was detected, with an estimated half-time for clearance of 160 days. No changes in fibre diameter distribution, or elemental content were observed with crocidolite. For chrysotile, there were 15-fold increases in the number of fibres in the lung over the 2-year study, due to fibre splitting. This was accompanied by a marked decrease in median diameter of the retained lung fibres. Leaching of magnesium from chrysotile fibres was much more distinct in the first month than in the following months.

3.16 For a special type of glass microfibre which had a low alkaline earth content, and for a ceramic wool, the half-times of clearance for fibres >5 µm in length were similar to those for crocidolite (3500 and 780 days). For another glass fibre with a high alkaline earth content, a half-time for clearance of 14 days was estimated. Other glass and rockwool samples had estimated half-times for clearance ranging from 55-283 days. There are some difficulties in comparing the pulmonary clearance rates of the different fibre samples due to differences in the fibre size distributions of the administered fibres. Solubility is likely to be influenced by both fibre length (as discussed in paragraph 3.24) and by fibre diameter (thinner fibres would be expected to dissolve more quickly than thicker ones).

3.17 Based on the results of lung fibre burden measurements in a 30-month inhalation study in baboons conducted by Rendall (1988) cited by Oberdorster (1994), the

pulmonary clearance half-time for chrysotile was judged to be about 90 days. However no information was given on the differential clearance rates for retained chrysotile fibres of different lengths. Thus the value of 90 days cited as a clearance half-time must be viewed as an 'average', with longer half-times for the longer length fibres to be expected, and more rapid clearance for the shorter ones. The pulmonary clearance rates for glass fibres, amosite and crocidolite measured in this study ranged between 200-1500 days. In long term inhalation studies in rats with different samples of asbestos (see Table 1) reported by Wagner *et al* (1974) the pulmonary burden of chrysotile reached a plateau within 3-6 months of exposure, whereas the pulmonary burdens of amphiboles continued to rise during the 2 years of exposure. Again, these results are limited in value by the lack of detailed information on the fibre size distributions of the retained lung fibres.

Interstitialisation

3.18 The passage of inhaled fibres across the alveolar epithelial barrier into the pulmonary interstitium has been called interstitialisation, (discussed in paragraph 5.16). The transfer of fibres into the pulmonary interstitium is thought to enhance the potential for pulmonary interstitial fibrosis development.

3.19 Within hours of a 1-hour nose-only inhalation exposure in rats, chrysotile fibres were observed to penetrate the pulmonary interstitium after being taken up by Type I epithelial cells (Brody *et al*, 1981). Scanning electron microscopy revealed that the respirable fraction of asbestos in the fibre aerosols contained fibres 95% of which were less than 6 µm in length, and no fibres greater than 0.5 µm in diameter. This transepithelial movement of fibres occurred without the involvement of AMs. Following penetration into the interstitium, chrysotile fibres were taken up by interstitial macrophages and fibroblasts. Chrysotile fibres were also observed in capillary endothelial cells and in capillary lumina adjacent to alveolar duct bifurcations.

3.20 Following penetration to the pulmonary interstitium, fibres may be transported to regional lymph nodes by macrophages. Macrophages containing short aramid fibres have been observed in the tracheobronchial lymph nodes in rats, indicating that following alveolar deposition aramid fibres are able to cross the epithelial barrier and move into the interstitium (Lee *et al*, 1988). Clearly, inhaled fibres can be translocated away from the initial sites of deposition to other regions.

Fibre translocation to the pleura

3.21 The mechanism of migration of inhaled fibres to the pleura is poorly defined. It has been suggested that fibres may reach the pleura through lymphatic drainage channels, by transport within interstitial macrophages, or by mechanical translocation through lung tissue (Oberdorster, 1994). In IT studies in mice with samples of long and short fibre crocidolite it was observed that the long crocidolite fibres became incorporated into the underlying connective tissue of the bronchial and bronchiolar epithelium, and in some sections of the lung, peribronchial or peribronchiolar interstitium could be traced directly to the pleural surface with no intervening alveolar spaces (Adamson *et al*, 1993). This suggests that after crossing the epithelial barrier, some fibres may occur in close proximity to the pleural tissues. There is no evidence on how fibres reach the peritoneal mesothelium.

3.22 As described in paragraph 3.6, there is evidence that over a period of months inhaled fibres tend to accumulate in sub-pleural regions of the lung.

3.23 Lapin *et al* (1991) observed a single silicon carbide whisker in foci of hyperplasia in the mesothelium of each of two rats exposed to these whiskers for 13 weeks. However, there is little published information from human autopsy studies for asbestos fibres in pleural tissue.

Clearance rates - dependence on fibre length

3.24 In a study by Morgan *et al* (1982), the pulmonary clearance of 1.5 μm diameter glass fibres of lengths 5 to 60 μm was measured following intratracheal administration in rats. After one year, 90% of the 5 μm fibres had been cleared, and 80 % of the 10 μm fibres. No significant clearance of the 30 or 60 μm fibres occurred within this time period. From these results it is clear that macrophage clearance is less effective for 10 μm length fibres than for 5 μm , and is ineffective for fibres of 30 μm and longer.

3.25 In a study by Coin *et al* (1992) rats were exposed for 3 hours to chrysotile aerosols, and were sacrificed at intervals up to 29 days post-exposure for analysis of the lung burdens of fibres. It was found that clearance half-lives increased with increasing fibre length: 10 days for fibres 0.5-4 μm , increasing to 114 days for fibres >16 μm . However, given the short duration of the study, it is unclear if dissolution of the retained chrysotile might have contributed substantially towards clearance in the longer term. Analyses of the length distribution of airborne anthophyllite fibres, and those found in the lungs of Finnish workers, suggested that 17 μm was the upper limit for macrophage-mediated pulmonary clearance of such fibres (Timbrell, 1982).

Fibre solubility

3.26 Some fibres may undergo disintegration or dissolution in the lung which will facilitate clearance. The dissolution of fibres in the lungs may occur by both surface attack and by leaching within the structure. It has been suggested that these processes may cause a weakening of the structure and possibly fragmentation into shorter segments (Lippmann, 1990). The shorter fragments may then be more readily cleared by macrophages.

3.27 Scholze and Conradt (1987) investigated the chemical durability of seven vitreous, three refractory and three natural mineral fibres in an *in vitro* study using physiological solution and flow through conditions. The silicon concentration of the leachate was used as a measure of network dissolution, and in some cases the effect of selective leaching of elements such as boron or potassium was also analysed.

3.28 The results showed that the MMMFs exhibited network dissolution velocities from 0.2 to 3.5 nm per day, whereas the values for chrysotile and crocidolite were 0.011 and 0.0002 nm per day respectively. The predicted times in which MMMFs of 1 μm diameter would completely dissolve ranged from 0.4 to 6.5 years, whereas the corresponding times for chrysotile and crocidolite were estimated to be about 100 years or more. Although these results suggest that chrysotile would be extremely durable in the lungs no information was provided on fragmentation of these fibres under the test conditions, nor was the loss of magnesium from chrysotile reported upon.

3.29 It is widely accepted throughout the literature on fibre toxicology that chrysotile is less durable in the lungs than amphibole forms of asbestos (Sebastien, 1991). For example, it has been shown that in the lungs of the Quebec miners and millers of chrysotile, the ratio of tremolite to chrysotile increases with increasing duration of exposure, despite the fact that tremolite is present as a very low percentage of the total airborne fibres (Churg, 1994). The shorter residence of chrysotile in the human lung compared with amphiboles has been attributed to the leaching of magnesium ions from chrysotile, which leads to a loss of structural integrity and fragmentation of the fibres (IPCS, 1988). In a study cited by Morgan (1994) the dissolution kinetics of chrysotile was examined and found to be a two-stage process, in which the initial step is the dissolution of magnesium, leaving behind the silicon which dissolves at a slower rate. From the results of this study it was predicted that a chrysotile fibre with 1 μm diameter will dissolve in the lung with a half-time of 9 months. At a recent international workshop on chrysotile it was agreed overall that while chrysotile is removed from the

lungs more effectively than amphiboles, some fibres are able to persist over a timescale of years (Morgan, 1994).

3.30 There is evidence that the solubility of glass fibres depends on the content of alkaline earths and earth oxides (Potter and Mattson, 1991). These authors measured the *in vitro* dissolution rates of 30 different glass fibre compositions. From the results, an empirical formula has been derived based on the mineral oxide composition which may be able to rank fibres in terms of increasing solubility:- Solubility Index = $\Sigma \text{Na}_2\text{O}, \text{K}_2\text{O}, \text{BaO}, \text{CaO}, \text{MgO}, \text{B}_2\text{O}_3 - 2 \times \text{Al}_2\text{O}_3$ (% by weight). This is consistent with the experimental evidence from the Bellman (1987) study which showed rapid lung clearance (presumably due to dissolution) of glass fibres with a low alkaline earth and earth oxide content.

3.31 McClellan *et al* (1992) suggested that durability is a crucial factor for determining the toxicity of mineral fibres and that both aspects increase in the following order: glass fibre < slag wool < rock wool < refractory ceramic fibre < chrysotile < amphibole asbestos. However, this does not acknowledge the substantial variation which can exist in the solubility of different glass fibre compositions, and that some glass fibre compositions can be very durable. When rats were exposed to approximately equal airborne concentrations of two different types of glass fibre, or a refractory ceramic fibre (RCF) for 2 years, at the end of the study there was no major difference in lung fibre burdens between the groups of rats (Bunn *et al*, 1993; Hesterberg *et al*, 1993). This suggests that in terms of durability, no distinction could be made between these fibres over the duration of these studies. However, in the rats exposed to RCF, pulmonary fibrosis, lung cancer and mesotheliomas occurred, whereas the two types of glass fibre produced no such effects. Therefore the observed differences in toxicity do not appear to be due to differences in *in vivo* durability, suggesting that other parameters such as surface chemistry, must be important determinants of toxicity.

3.32 The solubility of MMMFs in rat lungs is dependent on fibre length. Morgan *et al* (1982) administered doses of sized glass fibres to rats by IT instillation, and groups of animals were killed at intervals up to 18 months post-exposure. As the experiment progressed it became apparent that there was a reduction in the diameter of the fibres isolated from the lung, and the effect was more marked with the longer fibres. For glass fibres of 1.5 μm diameter, there was much less dissolution of the 5 and 10 μm fibres than of the 30 and 60 μm fibres. The dissolution of these fibres was non-uniform, some were little changed in dimension, while others were reduced in diameter to 0.2 μm . In addition, it was found that the count median lengths of the 60 x 1.5 μm fibres were reduced to about 40 μm after 9 months, and after 18 months to 25 μm . The authors suggested that these results were very different from those seen with amphibole asbestos fibres, which are virtually insoluble *in vivo*.

3.33 To explain the effect of fibre length on dissolution rates, the authors suggested that glass fibres are more susceptible to dissolution in the more alkaline extra-cellular fluid of the lungs, whereas short fibres contained within macrophages are exposed to a lower pH and are more resistant to leaching. Further evidence for this suggestion was reported by this group in an abstract from the Lyons (IARC) conference on fibre durability 1992. Although shorter fibres are more readily cleared by macrophages, these results indicate that in the long term, the dissolution of longer glass fibres in the lungs should contribute to pulmonary clearance.

Summary of deposition and clearance section

3.34 The regional deposition efficiency of inhaled fibres is largely a function of fibre diameter and density, with length and aspect ratio being of minor importance. Maximum alveolar deposition efficiency in humans is expected to occur with mineral

fibre diameters of about 1 μm , and to decrease with values above this, so that above 3 μm in diameter, fibres would be essentially non-respirable.

3.35 The predominant sites of pulmonary deposition of inhaled fibres are found around the junctions of terminal bronchioles and alveolar ducts, known as alveolar duct bifurcations. These are also the initial sites of development of pulmonary fibrosis caused by inhaled fibres.

3.36 Although the methods whereby inhaled fibres translocate to the pleural tissues are poorly understood, it would appear that the direct penetration of fibres across the alveolar epithelial barrier does occur (at least with some fibres). Furthermore, some peripherally located alveoli are very close (0.5 mm) to the pleura. This is suggestive of the possibility of direct penetration of fibres to the pleura. There is no evidence on how fibres reach the peritoneal mesothelium.

3.37 Clearance of fibres which deposit in the alveolar regions of the lung takes place by macrophage-mediated phagocytosis which is highly effective for short fibres (<5 μm), but becomes increasingly difficult with increasing fibre length, so that there will be negligible macrophage-mediated clearance for fibres of lengths around 16 μm or more. In the longer term, some fibres, depending on chemical composition, will undergo dissolution or leaching of particular elements from the fibre structure. This may lead to fragmentation into shorter lengths which will facilitate clearance. There is evidence that the pulmonary clearance of chrysotile is more rapid than for amphibole fibres of similar dimensions.

Section 4

ANIMAL STUDIES IN FIBRE TOXICITY

4.1 The aims of this section are to provide a framework for the interpretation of animal toxicity data with fibres in relation to different routes of administration, and to identify the relevance of these data to predicting fibre toxicity in humans. The conclusions reached in this section underpin the proposal for a fibre toxicity testing strategy (section 6), which is needed as a basis for hazard assessment of both new and existing fibre types.

4.2 Laboratory animals have been used since the 1930's to investigate the health effects of fibres. In 1951 Vorwald *et al* reported a long series of experiments which were begun in 1928. The results demonstrated that chrysotile asbestos produced peribronchiolar fibrosis of the lung similar to human asbestosis after inhalation or intra-tracheal (IT) injection in guinea pigs, rabbits and rats. Carcinogenic effects were not observed, but it is now clear that this was due to the generally shortened lifespan of laboratory animals at that time, caused by infections.

4.3 Vorwald (1951) showed that finely ground samples of asbestos did not cause pulmonary fibrosis in laboratory animals when instilled into the trachea, whereas smaller total amounts which contained long fibres were highly fibrogenic.

4.4 In 1974 Wagner *et al* reported upon inhalation experiments in SPF (specific pathogen free) rats which demonstrated the development of asbestosis, lung carcinomas and mesothelioma with different types of asbestos (amosite, anthophyllite, crocidolite, and chrysotile) (see Table 1). Such findings are not unique to asbestos; all these effects have also been produced in rats exposed by inhalation to four different types of RCF (Bunn *et al* 1993). Therefore, all of the diseases associated with asbestos in humans have been produced in inhalation studies in rats, both with asbestos and other fibre types.

Intra-pleural and intra-peritoneal administration

4.5 Since the 1970s investigations of fibre carcinogenicity have been conducted in rats using intra-pleural (IPL) and intra-peritoneal (IP) routes of exposure. Information from such studies has been of use in evaluating the role of fibre size and other fibre properties, and in investigating mechanisms of fibre toxicity.

4.6 IPL and IP administrations provide direct exposure of fibres to the target tissue (serosal membranes), and therefore can be said to maximise the sensitivity of the test. However these routes bypass the normal filtering mechanisms of the respiratory tract, and the delivery of a bolus dose of fibres may overwhelm the normal physiological clearance mechanisms. Thus fibres which might not necessarily elicit mesothelial effects following inhalation exposure, perhaps due to effective physiological clearance mechanisms and/or insufficient durability, may produce mesotheliomas when administered directly into the peritoneal or pleural cavity. Also, exposure via the IPL or IP routes does not allow an investigation of actual pulmonary responses to the test fibres.

4.7 Studies reported by Stanton and Wrench (1972), and Stanton *et al* (1977, 1981) involved the administration of different samples of asbestos, fibrous glass, non-crystalline silica and metal particles applied on gelatin-saturated coarse fibrous glass pledgets to the pleura of rats. Neither the pledget itself, nor the metal fragments caused pleural tumours, nor did the absence of the pledget alter the yield of crocidolite-induced pleural tumours. The amorphous silica induced 1 pleural tumour in 48 rats. Virtually all fibres tested in this way produced pleural tumours regardless of chemical composition.

Table 1 Summary of tumour findings from an inhalation study in rats with various types of asbestos (Wagner et al 1974)

Exposure 10-14 mg.m ⁻³	No. rats at risk	No. with adenoma	No. with adenomatosis	No. with adenocarcinoma	No. with squamous carcinoma	No. with mesothelioma
Amosite						
1 day	45	3	0	0	0	1
3 months	37	7	3	0	0	0
6 months	18	1	0	1	0	0
12 months	25	5	4	1	0	0
24 months	21	3	1	3	6	0
Anthophyllite						
1 day	44	2	0	0	0	0
3 months	37	6	0	0	0	0
6 months	18	3	1	1	1	0
12 months	28	9	6	4	1	1
24 months	18	2	5	3	6	1
Crocidolite						
1 day	43	5	0	1	0	1
3 months	36	10	2	1	1	1
6 months	18	2	2	0	0	0
12 months	26	5	4	3	6	2
24 months	18	4	5	2	2	0
Chrysotile (Canadian)						
1 day	42	9	0	1	0	0
3 months	34	15	0	3	0	0
6 months	17	2	2	0	1	0
12 months	23	1	3	6	1	3
24 months	21	2	3	1	4	1
Chrysotile Rhodesian						
1 day	45	4	0	1	0	0
3 months	36	11	2	3	0	0
6 months	19	2	3	3	0	0
12 months	27	2	4	7	6	0
24 months	17	0	1	5	5	0
Controls						
1 day	44	4	0	0	0	0
3 months	40	3	0	0	0	0
6-24 months	42	0	0	0	0	0
Total	126	7	0	0	0	0

4.8 From an analysis of the tumour findings in the Stanton studies in relation to the size distribution of the administered fibres (described in paragraphs 7.20-7.22), it was concluded that tumour development was most closely associated with fibres which were long ($>8 \mu\text{m}$) and thin ($<0.25 \mu\text{m}$), such fibres being known as 'Stanton fibres'. However, this conclusion may be open to question because comparisons were made on fibres which were applied in equal masses (40 mg), and not (as would be more appropriate) in equal numbers.

4.9 Since the 1970s a series of experiments in rats given IP injections of fibres has been reported by Pott *et al.* The peritoneal mesothelium was shown to be a sensitive target tissue for studying fibre-induced mesotheliomas. As with the IPL route of administration, almost all mineral fibres tested by this method (including glass fibres) have produced mesotheliomas, although certain very soluble fibres (eg wollastonite; Pott *et al* 1987) have not done so. Due to the high sensitivity of the IP test (occasional positives even with saline vehicle controls, tumour response rates of 0-10% with various non-fibrous dust samples), a tumour incidence of at least 10% is considered necessary by Pott to establish a 'positive' response.

4.10 For some synthetic organic fibres (eg aramid fibres), there is an added complication with IP injection studies. Davis *et al* (1987) and Pott *et al* (1987, 1989) have reported such studies with aramid fibres, and both workers found that the numbers of mesotheliomas produced were within vehicle control levels. However, it is difficult to obtain a stable homogeneous solution of such fibres, due to the marked tendency of the fibres to undergo agglomeration. The clumping of these fibres in solution markedly reduces the chances of individual fibre-cell interactions following injection.

4.11 There is a disparity between the positive results obtained in IPL and IP studies with glass fibres, and the consistently negative results for mesothelioma obtained in a number of inhalation studies (reviewed by IARC, 1988; IPCS, 1988). In addition, more recent inhalation studies in rats exposed to two different types of glasswool (Hesterberg *et al*, 1993) and to a glass microfibre (IOM unpublished data) also obtained negative results for mesothelioma.

4.12 Overall, in view of the comments made above (paragraph 4.5) the significant body of negative evidence with glass fibres in inhalation studies should be considered to outweigh the positive findings from IPL and IP studies. It is clear that positive results for mesothelioma in IPL and IP studies may well overestimate the hazardous properties of fibres when inhaled, towards the pleural membranes. However, due to the apparently high sensitivity of this test (with mineral fibres), negative results in an appropriately conducted IP test would strongly suggest an absence of hazard in relation to mesothelioma.

Intra-tracheal administration

4.13 IT studies involve the bolus delivery in the respiratory tract of what are usually relatively large numbers of fibres. It is recognised that the fibres would not be expected to deposit in a comparable fashion with inhalation exposure. There is no standardised method for delivery, different workers may deliver the instillation at different depths in the trachea, some workers may allow the animals to inspire to facilitate delivery, others may simply inject the material under pressure. Some workers may administer a single dose, others may give repeated injections. The lack of a validated or standardised method of IT administration leads to difficulties in comparing the results of different studies.

4.14 Oberdorster *et al* (1990) compared the acute inflammatory response caused by intra-tracheally instilled TiO_2 particles to the response produced by inhalation. The authors concluded that the delivered dose rate strongly influences the acute inflammatory response and the potential for interstitialisation. An equivalent dose given

over days by inhalation provoked a mild response compared with that elicited by single sudden instillation delivery. This reflects the importance of alveolar macrophages (AMs) in clearing the broncho-alveolar airway epithelium of deposited particles. A single massive dose will overwhelm macrophage clearance mechanisms, but a gradually administered dose will allow time for macrophage recruitment (dust cell response) which is a physiological adaptation to the presence of inhaled particles. Nevertheless, the instillation technique is a valuable tool to elucidate mechanisms of particle-induced lung injury.

4.15 Unfortunately, few IT studies have been conducted specifically to investigate potential carcinogenic responses to fibres and therefore the value of such studies is difficult to assess. Coffin *et al* (1992) reported the results of long term IT instillation studies in male rats with UICC chrysotile, UICC crocidolite and erionite (see para 4.24 for definition of UICC). All fibres produced increases in both lung carcinomas and mesotheliomas (see Table 2 for results). The dose response relationships for each fibre type were somewhat erratic, which may be a result of uneven deposition effects.

4.16 The results show that each dose of chrysotile (in terms of mg) produced more tumours than the equivalent dose of crocidolite. In fact the fibre numbers (measured as 'Stanton fibres' with lengths $>8 \mu\text{m}$ and widths $<0.25 \mu\text{m}$) at each dose level were orders of magnitude greater with chrysotile than crocidolite. Therefore the pattern of responses produced by these fibres is consistent with the evidence from human studies. Overall, the results suggest that IT studies with fibres may be useful in hazard identification.

Table 2 Tumour incidence in rats exposed to mineral fibres by intratracheal instillation (Results taken from Coffin *et al* 1992)

Fibre and tumours	Calculated accumulated dose (mg)					
	6 mg		16 mg		32 mg	
	No. of animals	Percent tumours *	No. of animals	Percent tumours *	No. of animals	Percent tumours *
Erionite						
-Mesothelioma	119	31.9	118	61	116	81
-Lung carcinoma	119	1.7	118	0.9	116	4.3
Chrysotile						
-Mesothelioma	132	8.3	133	10.5	41	9.8
-Lung carcinoma	132	27.3	133	14.3	41	2.4
Crocidolite						
-Mesothelioma	132	6.8	132	4.6	132	3.8
-Lung carcinoma	132	3.8	132	0.9	132	9.1
Controls						
-Mesothelioma	285	1.4	-	-	-	-
-Lung carcinoma	285	1.4	-	-	-	-

* Data represent animals dying with tumours

4.17 The few IT studies reported with glass fibres have produced conflicting results. The IT injection of 10 mg (20 weekly doses of 0.5 mg) of glass fibre produced 4 lung carcinomas and 1 adenoma in a group of 34 rats (Pott *et al*, 1987). No lung tumours were produced by glass fibres in a similar study by Smith *et al* (1987). IT administrations of two glass fibre samples (8 weekly doses of 1 mg) in hamsters produced lung tumours (up to 3.7%) as well as high incidences of mesothelioma (up to 27%) in one study (Mohr *et al*, 1984). The titanium dioxide granular dust used as a negative control in this study did not produce any lung carcinomas or mesotheliomas

when given in the same gravimetric dose. However, no thoracic tumours were produced by glass fibres in a similar study in hamsters reported by Feron (1985) (summarised in IARC, 1988).

4.18 In addressing the role of IT studies on fibres and the significance of their findings, it seems sensible to conclude that positive carcinogenicity findings in an IT study give rise to more concern than positive results in an IP test, as the route of administration is more closely related to inhalation exposure. However, a consistently negative set of results for lung cancer and mesothelioma has emerged with glass fibres in numerous animal inhalation studies which raises the question of whether or not IT studies also overestimate the hazardous properties of fibres when inhaled. The most appropriate scientific position to adopt seems to be to regard positive carcinogenicity findings in an IT study as valid evidence of carcinogenic potential for inhaled fibres unless counteracted by negative results from one or more well conducted inhalation studies. In contrast, negative results from a well conducted IT study would suggest an absence of hazard, and in the context of a fibre toxicity testing strategy, a move to inhalation carcinogenicity testing would not be justified.

Inhalation studies

4.19 The inhalation route is of most relevance to human exposures to fibres and therefore animal studies using this exposure route should provide the the most appropriate basis for hazard identification and for investigating dose-response relationships.

4.20 At a WHO working party which discussed the importance of the route of administration (Copenhagen, 1992) it was concluded that 'properly conducted inhalation studies in rats, using fibres of appropriate size, are adequately sensitive for estimating human hazards for lung cancer and interstitial fibrosis from inhaled fibres'. It is proposed here that this is the most appropriate scientific position to adopt. However, note that mesothelioma effects are not included in the WHO working party statement.

4.21 Evidence on the role of fibre size in relation to toxicity obtained in animal inhalation studies is summarised in paragraphs 7.29-7.32.

4.22 Currently, there is a lack of a validated and internationally accepted protocol for inhalation carcinogenicity studies with fibres. For example, standard carcinogenicity studies in rats (eg according to OECD Guidelines) require that the terminal sacrifice should take place at 24 months from the start of exposure. However, mesothelioma development is rarely seen in a rat before 28-30 months from the start of exposure. Standard carcinogenicity studies usually require 50 animals per sex per dose. However, many fibre inhalation carcinogenicity studies have been carried out in male rats only, and no adequate justifications have been offered. There seems to be no reason why both sexes should not be examined.

4.23 Potentially, inhalation experiments allow investigation of all of the end-points of concern - pulmonary fibrosis, lung cancer, and also mesothelioma. The adequacy of inhalation studies in rodents for detecting fibre-induced mesothelioma is discussed below.

Choice of species in inhalation studies

4.24 Most of the available studies (results given in Table 3) used standard samples of asbestos which were collected in 1966 under the auspices of the Union Internationale Contre le Cancer (UICC). These were provided to allow valid inter-laboratory comparisons, and had been subjected to extensive ball-milling to ensure the respirability of the fibres. This resulted in a shortening of fibre lengths, so that the mean fibre length in these samples was only about 2 μ m (Donaldson *et al*, 1993). The short fibre lengths

of the reference UICC asbestos samples may account for the low tumour incidences observed in animal studies.

4.25 However, recently, a long fibre sample of crocidolite was used as a positive control in a rat inhalation study (McConnell *et al*, 1994). Due to toxicity, exposure was terminated at 10 months into the study. Animals were maintained until the 20% survival time. The fibre aerosol count was 1610 f/ml >5 µm in length, containing 236 f/ml >20 µm. Only one mesothelioma was found in 106 rats, together with a 6% incidence of lung carcinoma. Thus exposure to a known human mesothelial carcinogen, which would presumably equate to a prolonged and extremely heavy exposure in human terms, produced less than a 1% incidence in mesothelioma in rats.

4.26 In contrast, in an early rat inhalation study, (see Table 1), one rat each from groups exposed to amphibole asbestos samples for only 1 day developed mesotheliomas (Wagner, 1974).

4.27 From this pattern of evidence no firm conclusions can be drawn about the relative susceptibility of the rat and human to asbestos-induced mesothelioma. Any meaningful comparison of the susceptibility of different species must take into account differences in exposure concentrations and fibre size distributions. Such detailed quantitative information is not available for the human evidence.

4.28 In rat inhalation studies with RCFs, exposure of large group sizes (>120) to four different types of RCF at 200 f/ml (~20 µm in length) produced up to 2.5% mesotheliomas and 8% lung carcinomas (Bunn *et al*, 1993). Also, a high incidence (almost 100%) of mesotheliomas occurred in rats exposed to erionite (Wagner *et al*, 1986). Erionite has also been shown to be markedly more active than crocidolite in producing mesothelioma when given by the IT route (Coffin *et al*, 1992). Overall, it can be concluded that the rat will develop fibre-induced lung carcinomas and mesotheliomas in inhalation studies. However, for the reliable detection of the potential to produce mesothelioma, large group sizes of rats (>100) are needed.

4.29 The hamster has been used in a limited number of fibre carcinogenicity studies. However, this species appears to be resistant to the development of lung tumours induced by inhaled solid particles. For example, following exposure to 200 f/ml of RCF, 0/102 hamsters but 8/123 rats developed lung carcinomas (Bunn *et al*, 1993). Hamsters also appear to be resistant to the development of lung tumours when exposed to other known lung carcinogens ie cadmium and nickel (HSE Toxicity Reviews Nos 24 and 17).

4.30 Interestingly, the hamster may be relatively susceptible to the development of mesothelioma. In the above study, 42% of hamsters but only 1-2% of rats developed mesothelioma under similar exposure conditions. However, only one or two of the hamster mesotheliomas were clearly detectable diffuse mesotheliomas (by macroscopic observation). The majority of the hamster mesotheliomas were microscopic in size, and were searched for with a rigour not previously applied in inhalation carcinogenicity studies on fibres. This raises a number of questions, including the possibility that such tumours may have occurred in previous studies in rats but went unnoticed. No confirmatory studies have yet been conducted to compare the responses of hamsters and rats, and therefore no absolutely definite conclusions can yet be drawn about the relative sensitivity of these species in fibre inhalation studies.

Evidence for a relationship between pulmonary fibrosis and lung cancer

4.31 In a long-term rat inhalation study using different types of asbestos a relationship was observed between the incidence of lung cancer and the development of pulmonary fibrosis, in that there was far more pulmonary fibrosis in animals with lung tumours than in animals without tumours (Wagner, 1974).

4.32 This relationship has been noted in other animal studies with asbestos and with MMMFs (Davis and Cowie, 1990). These authors reviewed the histological slides from a number of fibre inhalation studies. They found that animals with pulmonary tumours had almost double the amount of pulmonary fibrosis as animals of a similar age without tumours. In animals where tumours were found at an early stage of development, their origin from fibrotic areas could be confirmed. As described in a later review, fibre-induced pulmonary tumours in rats 'usually occur in areas with the most severe asbestosis where epithelial hyperplasia and metaplasia are common' (Davis, 1991).

4.33 In a recent study with RCFs (see Table 4) the results again suggest that there will be no increased risk of lung carcinoma at exposures which do not cause pulmonary fibrosis. This is part of the evidence used to support the contention that fibre-induced lung cancer occurs only in the dose-range at which fibrosis is also produced (refer to paragraphs 2.35 - 2.39).

Knowledge of dose-response relationships from inhalation studies with fibres

4.34 No adequately conducted animal inhalation experiments have been performed to investigate dose-response relationships with any of the asbestos types, nor have any of the asbestos studies allowed a clear identification of no-observed adverse effect levels (NOAELs) for any of the end-points of concern.

4.35 In the Wagner study of 1974, rather than vary the exposure concentrations, rats were exposed for varying periods of time ranging from 1 day to 24 months. A clear and consistent trend of increasing tumour numbers with increasing durations of exposure did not emerge, although this may have been due to the small group sizes of animals used (see Table 1).

4.36 Although there are no inhalation studies which have investigated dose-response relationships with asbestos, some useful information is available from a study with RCF. These results are presented in Table 4.

Table 4 Summary of tumour findings from an inhalation study in rats with a refractory ceramic fibre (Bunn et al, 1993)

Kaolin-based RCF Mg/m ³	Fibres/ml	No. of rats at risk	No. of adenoma	Pulmonary interstitial fibrosis	No. of lung carcinoma	No. of mesothelioma
Air 0	0	129	1	No fibrosis at any time	0	0
3	25	123	2	Macrophage response but no significant fibrosis	0	0
9	75	127	4	Irreversible fibrosis by 12 months	1	1
16	115	124	1	Irreversible fibrosis by 12 months	1	0
30	200	123	8	Irreversible fibrosis by 6 months	8	2

4.37 From these results a no-effect level for pulmonary fibrosis and lung cancer can be identified at 25 f/ml. No mesotheliomas were seen at this exposure level, although as discussed later (paragraph 4.41) this observation should be interpreted cautiously.

4.38 In a 2-year inhalation study employing multiple exposure concentrations of aramid fibres, a clear no-effect level for pulmonary toxicity of 2.5 f/ml was detected (Lee et al, 1988). Above this concentration there were dose-related increases in pulmonary toxicity, although at the next higher exposure concentration of 25 f/ml, only slight evidence for pulmonary toxicity was observed. At concentrations above this there

was marked cytotoxicity particularly to the epithelium of the terminal bronchiolar and alveolar airways.

4.39 The evidence from animal inhalation studies with synthetic fibres suggests that the production of pulmonary toxicity is a threshold phenomenon.

4.40 As part of the RCF studies described above, rats were exposed to 10 000 f/ml of chrysotile as a positive control. In these rats, irreversible pulmonary interstitial fibrosis was evident within 3 months of exposure, with a subsequent incidence of lung carcinoma of 9%. Thus, based on the results from the RCF and chrysotile exposures, it is possible that histopathological detection of irreversible pulmonary fibrosis within 3-6 months of exposure may act as a marker for the subsequent development of lung cancer.

4.41 Although the lowest dose level used in the RCF study did not produce mesotheliomas, it should be noted that there was a rather low incidence and inconsistent pattern for this tumour throughout the study. Given the inconsistent pattern and small numbers of RCF-induced mesothelioma, no firm conclusions about the existence of a no-effect level for this end-point can be drawn.

Fibre aerosol concentrations in rat inhalation studies

4.42 There might be some question regarding the relevance of tumour findings in a rat inhalation study where concentrations of hundreds of fibres/ml are used. Human autopsy evidence indicates that high lung burdens of fibres are necessary for the development of asbestosis and lung cancer, requiring lung burdens of at least 5×10^5 fibres/dried gram of lung (fibres $>5 \mu\text{m}$ in length) (Davis and Donaldson, 1993). However, a human might have 20 years or more in which to accumulate such high lung burdens, whereas a rat can only be exposed for about 2 years.

4.43 It therefore seems justified, that in order to rigorously investigate fibre toxicity, and in view of the inherent limitations imposed on the sensitivity of the assay (minimum observable risk of about 1/100) that rats should be exposed to aerosol concentrations which are sufficiently high to produce lung fibre burdens comparable to those found in human cases of asbestosis and lung cancer. Davis and Donaldson 1993 suggested that in order to achieve adequate lung fibre burdens in rats, animals should be exposed for at least 12 months to dust cloud concentrations of at least 100 and preferably 200 fibres/ml (fibres $>20 \mu\text{m}$ in length).

Summary of animal studies in fibre toxicity

4.44 Different routes of administration have been used in animal studies with fibres. IPL and IP administrations provide direct exposure of fibres to the target tissue (mesothelial membranes), and therefore can be said to represent maximum sensitivity as regards to a test for the capacity to produce mesothelioma; these routes are not helpful in investigating lung effects. IPL and IP studies appear to be overly sensitive in relation to mesothelioma, in the sense that fibres which perhaps because of effective lung clearance mechanisms do not pose a hazard by the inhalation route, can elicit mesothelioma when instilled directly into the pleural or peritoneal cavities. In view of this, negative results for mesothelioma induction by mineral fibres in a well conducted IPL or IP study would suggest an absence of carcinogenic hazard towards the mesothelial tissues. IPL and IP studies may be inappropriate for investigating organic fibres, because the physical properties of these fibres preclude the ability to inject a homogeneous solution. In this case negative data may be unreliable.

4.45 IT instillation, whilst addressing the same route of administration as inhalation, does not mimic the pattern of pulmonary deposition for inhaled fibres. The same dose delivered as a single IT bolus can elicit a more severe inflammatory response in the

lungs than when delivered gradually by inhalation. Therefore, for the purpose of evaluating potential human health effects of fibres, small doses given repeatedly, perhaps once or twice weekly over many months, should provide the most reliable results with this route of delivery.

4.46 It would seem sensible to regard positive carcinogenicity findings in an IT study as valid evidence of carcinogenic potential for inhaled fibres unless counteracted by negative results from one or more well conducted inhalation studies. In contrast, negative results from a well conducted IT study would suggest an absence of hazard, and in the context of a fibre toxicity testing strategy, a move to inhalation carcinogenicity testing would not be justified.

4.47 The inhalation route is of most relevance to human exposures to fibres and therefore animal studies using this exposure route should provide a clearer basis for hazard identification and for investigating dose-response relationships. Inhalation studies in rats are able to demonstrate the known human health hazards of asbestos. However, in general, very few mesotheliomas can be produced with this exposure route, therefore large group sizes of rats (>100) are needed to reliably identify this end-point.

4.48 Animal studies with RCFs and with asbestos suggest a relationship between the development of pulmonary fibrosis and lung cancer, in that lung tumours are only seen at exposures which are sufficient to produce pulmonary fibrosis. Furthermore, histopathological evidence suggests that the early appearance of irreversible pulmonary fibrosis (eg 3-6 months) in a fibre inhalation study may act as a marker for the subsequent development of lung cancer.

4.49 Results with RCFs provide evidence for the existence of a no effect level for pulmonary fibrosis and lung cancer, and therefore provide along with other lines of evidence, support for a threshold model for these diseases. A threshold for mesothelioma cannot be reliably identified from the animal inhalation studies with asbestos or RCFs. No experimental evidence for a threshold for the development of mesothelioma is available.

Table 3

LIFETIME INHALATION STUDIES WITH AMPHIBOLE ASBESTOS

Fibre Type	Exposure	Comments	Benign Lung Tumours and non-tumour pathology	Malignant Lung Tumours	Mesothelioma	Reference
UICC crocidolite (see table 1 for further details)	10-12 mg/m ³ Information on fibre size distribution not provided	Groups of between 19 and 49 Wistar rats were exposed for periods of between 1 day to 24 months, and were then maintained for the remainder of their lifespan.	Numbers of adenomas ranged between 2 and 10 per group, with no clear relation to duration of exposure. The degree of pulmonary fibrosis increased with increasing duration of exposure.	Numbers of adenocarcinomas ranged between 0-3 per group, and squamous carcinomas were found in 8/26 rats from the 12 month exposure group.	One mesothelioma was found in a rat from the 1-day and the 3 month exposure groups. Mesothelioma were found in two rats from the 12 month exposure group.	Wagner et al (1974)
UICC crocidolite	10 mg/m ³ 1630 f/ml >5µm	20 male and 20 female Fischer 344 rats were exposed for 12 months and then allowed to live out their lives	Non-tumour pathology not specifically discussed in the report.	1 sq. carcinoma among 28 rats	0	Wagner et al (1985) (positive control in a study with erionite).
UICC crocidolite	10 mg/m ³	20 male and 20 female Fischer rats were exposed for 12 months and were then maintained for their lifespan.	3/28 rats with bronchoalveolar hyperplasia. Only in rats killed at the 3-month interim kills was there any fibrosis reaching Grade 4 on the Wagner scale.	1 adenocarcinoma among 28 rats.	0	Wagner et al (1987) (positive control in a study with pyrogenic dusts).
South African crocidolite (slightly longer than UICC crocidolite)	2.2 mg/m ³ Total fibre concentration = 2011 f/ml of which 162 f/ml >5µm	50 female Wistar rats were exposed nose-only 5 hr/day, 4 days/week, for 12 months. Median lifespan was 111 weeks.	A high incidence of broncho-alveolar hyperplasia was found in crocidolite exposed rats. The degree of pulmonary fibrosis was slight to medium.	1 adenocarcinoma among 50 rats	0	Muhle et al (1987) (positive control in a glass fibre study).
UICC crocidolite	10 mg/m ³ 860 f/ml >5µm 34 f/ml >20 µm	48 SPF rats of the Han strain were exposed 7 hrs/day, 5 days/week for 12 months, and were then maintained for their lifespan.	Peribronchiolar and interstitial fibrosis, and bronchiolisation. 1 adenoma in 40 rats	0 in 40 rats	0	Davis et al (1978)
UICC crocidolite	5 mg/m ³ 430 f/ml >5µm 17 f/ml >20 µm	48 Han rats were exposed 7hrs/day, 5 days/week, for 12 months and were then maintained for their lifespan.	As above, with peribronchial and interstitial fibrosis, and bronchiolisation	0 lung tumours in 43 rats	1 pleural mesothelioma in 43 rats	Davis et al (1978)

Table 3

LIFETIME INHALATION STUDIES WITH AMPHIBOLE ASBESTOS

Fibre Type	Exposure	Comments	Benign Lung Tumours and non-tumour pathology	Malignant Lung Tumours	Mesothelioma	Reference
UICC crocidolite	7 mg/m ³ 3000 f/ml of which 97% < 5 µm in length. 80 f/ml > 10 µm	Male Syrian hamsters and female Osborne Mendel rats were exposed nose-only 6 hr/day, 5 days/week, for 24 months and then maintained for their lifespan. Only 3/58 hamsters and 27/57 rats survived to 24 months.	Mild pulmonary fibrosis was seen in both species with a higher frequency in rats. Some broncho-alveolar hyperplasia was seen and this was more frequent in hamsters.	2 broncho-alveolar tumours were seen in 57 rats. No lung tumours were seen in hamsters.	1 mesothelioma was seen in 57 rats	Smith et al (1987)
NIEHS crocidolite	10 mg/m ³ 1610 f/ml > 5 µm 667 f/ml > 10 µm 236 f/ml > 20 µm	140 male Fischer rats exposed nose-only 6 hrs/day, 5 days/week, for 10 months. Maintained until 20% survival which occurred at 28 months.	Labouring breathing and premature mortality occurring at 9 months led to termination of exposures at 10 months. Extensive pulmonary pathology including metaplasia of alveolar epithelium, bronchiolisation, peribronchial and interstitial fibrosis. Minimal to mild focal fibrosis of visceral pleura in majority of rats. 10/106 rats with pulmonary adenomas.	6/106 rats with pulmonary carcinomas	1/106 rats with a pleural mesothelioma	McConnell et al (1984) Used as a positive control in a study with rock and slag wool.
Long fibre amosite (South African)	10 mg/m ³ 2060 f/ml > 5 µm 1110 f/ml > 10 µm	48 male Wistar rats exposed 7 hrs/day, 5 days/week for 12 months and then maintained for the rest of their lifespan.	3 adenomas in 40 rats. Deposits of granulation tissue, peribronchial and interstitial fibrosis, thickening of alveolar septa.	8 pulmonary carcinomas in 40 rats	2 pleural mesotheliomas in 40 rats	Davis et al (1986)
Short-fibre amosite (South African)	10 mg/m ³ 70 f/ml > 5 µm 12 f/ml > 10 µm	As above	No evidence of peribronchial fibrosis. Interstitial fibrosis as in aged control rats	0 pulmonary tumours	No mesothelioma	Davis et al 1986
UICC amosite	10 mg/m ³ 550 f/ml > 5 µm 6 f/ml > 20 µm	As above	Thickening of alveolar septa, peribronchial and interstitial fibrosis, bronchiolisation. 2 adenomas in 43 rats.	No pulmonary carcinomas in 43 rats	No mesothelioma in 43 rats	Davis et al 1978

Table 3

LIFETIME INHALATION STUDIES WITH AMPHIBOLE ASBESTOS

Fibre Type	Exposure	Comments	Benign Lung Tumours and non-tumour pathology	Malignant Lung Tumours	Mesothelioma	Reference
UICC amosite	10-14 mg/m ³ Information on fibre size distribution not provided	Groups of between 21 and 48 Wistar rats were exposed for periods of between 1 day and 24 months, and were then allowed to live out their lifespan.	Numbers of pulmonary adenomas ranged between 1-7 per group with no clear relation to duration of exposure. Pulmonary fibrosis increased in severity with increasing duration of exposure.	6 squamous carcinomas and 3 adenocarcinomas were found in the 21 rats exposed for 24 months. 1 adenocarcinoma was found in 18 and 25 rats from the 6 and 12 month exposure groups respectively.	One mesothelioma in the 45 rats exposed for one day only.	Wagner et al (1974)
UICC anthophyllite	10-13 mg/m ³ Information on fibre size distribution not provided	Groups of between 19 and 49 rats were exposed for between 1 day and 24 months, and were then maintained for the remainder of their lifespan.	Numbers of pulmonary adenomas ranged between 2-9 per group. Pulmonary fibrosis increased in severity with increasing duration of exposure.	1/18, 4/28 and 3/18 rats from the 6, 12 and 24 month exposure groups with an adenocarcinoma. 1/18, 1/28 and 6/18 rats from these groups with squamous carcinomas.	1 mesothelioma in the 12 and 24 month exposure groups.	Wagner et al (1974)

Table 3 continued

LIFETIME INHALATION STUDIES WITH CHRYSOTILE ASBESTOS

Abbreviations: A = adenoma AC = adenocarcinoma SC = squamous cell carcinoma M = mesothelioma (p) = pleural (p) = peritoneal (exposures were for 6-7 hours/day, 5 days/week except where stated)

Fibre type	Exposure mg/m ³ f/cc > 5 µm	Comments	Benign lung tumours and non-tumour pathology	Malignant tumours	Reference
UICC Rhodesian chrysotile	10 1950 of which 360 f/cc > 20 µm	12 month exposure 29 month sacrifice	Extensive peribroncholar and interstitial fibrosis Bronchiolisation	6/40 (15%) AC 2/40 (5%) SC 0/40 M	Davis et al (1978)
UICC Rhodesian chrysotile	2 390 of which 72 f/cc > 20 µm	12 month exposure 29 month sacrifice	Peribroncholar and interstitial fibrosis Bronchiolisation	1/42 (2%) AC 1/42 (2%) SC 1/42 (2%) M (p)	..
Long fibre Canadian chrysotile	10 5510 of which 1950 f/cc > 10 µm 670 f/cc > 20 µm 320 f/cc > 30 µm	12 month exposure 31 month sacrifice The long fibre preparation produced much more peribroncholar and interstitial fibrosis than the short fibre preparation	6/40 (20%) A	6/40 (15%) AC 5/40 (12.5%) SC 2/40 (5%) M(p) 1/40 (2.5%) M(p)	Davis and Jones (1988)
Short fibre Canadian chrysotile	10 1170 of which 330 f/cc > 10 µm 33 f/cc > 20 µm 4 f/cc > 30 µm	12 month exposure 31 month sacrifice	1/40 (2.5%) A	6/40 (15%) AC 1/40 (2.5%) M (p)	..

Table 3 continued

LIFETIME INHALATION STUDIES WITH CHRYSOTILE ASBESTOS

Abbreviations: A = adenoma AC = adenocarcinoma SC = squamous cell carcinoma M = mesothelioma (pl) = pleural (pt) = peritoneal (exposures were for 6-7 hours/day, 5 days/week except where stated)

Fibre type	Exposure mg/m ³ f/cc >5 µm	Comments	Benign lung tumours and non-tumour pathology	Malignant tumours	Reference
UICC Canadian chrysotile	10	Positive control in MMMF study 12 month exposure Autopsy at end of lifespan, mean survival not clearly specified	Minimal fibrosis developed within 12 months which showed some signs of progression in animals sacrificed at later times 5/48 (10%) with broncho-alveolar hyperplasia 1/48 (2%) A	11/48 (23%) AC 0/48 M	Wagner et al (1984)
UICC Canadian chrysotile	10	presumably as above	Minimal fibrosis developed within 12 months and was equally severe in males and females 5/56 (9%) with broncho-alveolar hyperplasia 4/56 (7%) A	7/56 (12.5%) AC 0/56 M Pleural invasion by peripheral tumours was frequently observed	McConnell et al 1984
Canadian chrysotile	15	about 6000	Small focal lesions of septal fibrosis 9/47 (19%) with broncho-alveolar adenomatosis	No malignant tumours but very few animals remaining at end of study	Le Bouffant et al (1984)
Superfine chrysotile	10.8	about 500 12 month exposure 24 month sacrifice	3/21 (14%) A 2/21 (9.5%) with adenomatosis	1/21 (5%) AC	Wagner et al (1977)

Table 3 continued

LIFETIME INHALATION STUDIES WITH CHRYSOTILE ASBESTOS

Abbreviations:- A= adenoma AC= adenocarcinoma SC= squamous cell carcinoma M= mesothelioma (pl)= pleural (pt)= peritoneal (exposures were for 6-7 hours/day, 5 days/week except where stated)

Fibre type	Exposure mg/m ³ floc >5 µm	Comments	Benign lung tumours and non-tumour pathology	Malignant tumours	References
California chrysotile	6 131	Other studies with this type of chrysotile have not been reported. Nose-only exposure (exposures were only for 5 hours/day, 4 days/week) 12 month exposure Autopsy at end of lifespan, mean survival of 109 weeks	21/50 (42%) with septal interstitial fibrosis and/or interstitial inflammation 6/50 (12%) with broncho-alveolar hyperplasia No adenomas were reported	0/50	Muhle et al (1987)
Canadian NIEHS chrysotile	10 10,000 High proportion of fibres <5 µm in length, average lengths and diameters of fibres were 2.2 and 0.1 µm respectively	Positive control in RCF study at the RCC laboratories Nose only exposures with "state of the art" technology 24 month exposure 30 month sacrifice	Fibrosis developed within 3 months of exposure and did not progress much in severity throughout the study 7/69 (10%) A	6/69 (8%) AC 1/69 (1.4%) M	Hesterberg et al (1993)

Table 3 continued

LIFETIME INHALATION STUDIES WITH CHRYSOTILE ASBESTOS

Abbreviations:- A = adenoma AC= adenocarcinoma SC= squamous cell carcinoma M= mesothelioma (pl)= pleural (pt)= peritoneal (exposures were for 6-7 hours/day, 5 days/week except where stated)						
Fibre type	Exposure mg/m ³	Exposure f/oc >5 µm	Comments	Benign lung tumours and non-tumour pathology	Malignant tumours	Reference
UICC Canadian chrysotile	10	not reported	12 month exposure Autopsy at end of lifespan, mean survival 778 days from onset of exposure Survival unchanged of controls	Asbestosis developed within 3 months of exposure, and was more severe with the Canadian chrysotile than with the Rhodesian 1/23 (4%) A 3/23 (12%) with adenomatosis	6/23 (24%) AC 1/23 (4%) SC 3/23 (12%) M (pl)	Wagner et al (1974) (see Table 1 for further details)
UICC Canadian chrysotile	10	not reported	24 month exposure Autopsy at end of lifespan, mean survival 585 days from onset of exposure Survival less than controls	Non-tumour pathology as above. 20/21 (95%) A 3/21 (14%) with adenomatosis	1/21 (5%) AC 4/21 (20%) SC 1/21 (5%) M (pl)	Wagner et al (1974) (see Table 1 for further details)
UICC Rhodesian chrysotile	10	not reported	12 month exposure Autopsy at end of lifespan, mean survival 826 days from onset of exposure Survival unchanged compared with controls	Non-tumour pathology as above 2/27 (7%) A 4/27 (15%) with adenomatosis	7/27 (25%) AC 6/27 (22%) SC 0/27 M	Wagner et al (1974) (see Table 1 for further details)

Table 3 continued

LIFETIME INHALATION STUDIES WITH CHRYSOTILE ASBESTOS

Abbreviations: A = adenoma AC = adenocarcinoma SC = squamous cell carcinoma M = mesothelioma (pl) = pleural (pi) = peritoneal (exposures were for 6-7 hours/day, 5 days/week except where stated)

Fibre type	Exposure mg/m ³ f/cc >5 µm	Comments	Benign lung tumours and non-tumour pathology	Malignant tumours	Reference
UICC Rhodesian chrysotile	10 not reported	24 month exposure Autopsy at end of lifespan, mean survival 758 days from onset of exposure Survival unchanged compared with controls	Non-tumour pathology as above 0/17 A 1/17 (6%) with adenomatosis	5/17 (30%) AC 5/17 (30%) SC 0/17 M	Wagner et al (1974) (see Table 1 for further details)
UICC Chrysotile A (normal sample)	10 2650	12 month exposure 28 month final sacrifice Most animals developed vesicular hyperplasia of the pleural surfaces	Granulomatous response around terminal and respiratory bronchioles Peribroncholar and pulmonary interstitial fibrosis Thickening of alveolar septae with Type II pneumocyte hyperplasia 6/36 (17%) A	4/36 (11%) AC 4/36 (11%) SC 0/36 M	Davis et al (1988)
UICC Chrysotile A (electrostatically discharged)	10 2670	12 month exposure 28 month final sacrifice Most animals developed vesicular hyperplasia of the pleural surfaces	Granulomatous response around terminal and respiratory bronchioles Peribroncholar and interstitial pulmonary fibrosis Thickening of alveolar septa with Type II pneumocyte hyperplasia 4/39 (10%) A	4/39 (10%) AC 2/39 (5%) SC 1/39 (2.5%) M(pl)	Davis et al (1988)

Table 3 continued

LIFETIME INHALATION STUDIES WITH CHRYSOTILE ASBESTOS

Abbreviations:- A = adenoma AC = adenocarcinoma SC = squamous cell carcinoma M = mesothelioma (pl) = pleural (pt) = peritoneal (exposures were for 6-7 hours/day, 5 days/week except where stated)

Fibre type	Exposure mg/m ³ f/cc >5 µm	Comments	Benign lung tumours and non-tumour pathology	Malignant tumours	Reference
Chrysotile Type 7TF1	1 the count median length of the fibres was only 0.67 µm	18 month exposure 24 month sacrifice This chrysotile preparation had been subjected to prolonged heating and ball milling	No treatment-related pulmonary lesions were observed	0/38	Platak et al (1985)

Section 5

MECHANISMS OF FIBRE TOXICITY

5.1 The benefit in understanding the mechanisms of fibre toxicity lies in being able to predict the hazardous properties and perhaps some aspects of dose-response characteristics of existing and new fibre types; obviously these are very important considerations in risk assessment. Furthermore, an understanding of mechanisms of fibre-induced toxicity at a cellular and biochemical level is necessary for the design of short-term tests for evaluating fibre toxicity.

5.2 The possible mechanisms of fibre-induced toxicity have been discussed in a number of recent review papers (Rom *et al*, 1991; McClellan *et al*, 1992; Brown *et al*, 1990; Petruska *et al*, 1991; Mossman, 1993). Other relevant information is given in a recent series of review articles on basic mechanisms in lung disease and on particulate-induced lung tumours (Macnee and Selby, 1993; Sheppard and Harrison, 1992; Donaldson *et al*, 1992; Hext, 1994), and in a recent dosimetric modelling paper (Tran *et al*, 1994). The account given below is largely a synthesis of the information given in these reviews.

Mechanisms of lung injury leading to pulmonary fibrosis

5.3 Most current theories on the pathogenesis of pulmonary fibrosis suggest that this disease results from initial injury to alveolar and bronchiolar epithelium. Inflammatory cells then move into the alveolar spaces and interstitium and release mediators that stimulate collagen production by fibroblasts. Details of the cellular mechanisms involved in particle-induced lung injury, with particular emphasis on fibres, are given below.

Alveolar macrophage involvement

5.4 The function of alveolar macrophages (AMs) is to keep the alveolar airway passages clear by removing inhaled particles. This is achieved by phagocytosis and transport of particles towards the trachea for clearance from the lung via the mucociliary escalator.

5.5 AMs detect the presence of inhaled fibres either by chance during normal scavenging activities, or are recruited to the sites of fibre deposition following fibre-induced generation of C5a, a chemotactic factor derived from complement components contained within lung lining fluid. AMs have evolved for the purpose of killing microbial pathogens, and therefore phagocytosis is accompanied by the release of proteases and active oxygen species, which may cause injury to adjacent alveolar and bronchiolar epithelial cells.

5.6 Some inhaled particles (eg quartz) are directly cytotoxic to AMs, and the phagocytosis of quartz particles can cause necrosis of these cells. As these particles are released from necrotic AMs they will be rephagocytosed by additional AMs which will again undergo necrosis, and so the cycle will be repeated. During these activities there will be an increased secretion of inflammatory mediators and chemotactic agents from the particle laden AMs which will lead to inflammation and lung injury associated with an influx of phagocytes.

5.7 In contrast, other particles may not be directly cytotoxic, eg titanium dioxide, but if present in excessive amounts can 'overload' the normal macrophage clearance mechanism described above. In this context, 'overload' refers to the loss of mobility of AMs when excessive numbers of particles are phagocytosed (Morrow, 1988; 1992). In these situations, normal macrophage mediated clearance rates are inhibited; the balance between deposition and clearance will be affected such that there will be a non-linear increase in the lung burden of particles with continuing exposure. The overload phenomenon is associated with inflammation because the particle-laden AMs cannot

migrate effectively to the mucociliary escalator, but instead remain in the alveolar regions where they continue to secrete inflammatory and chemotactic mediators.

5.8 The process of AM-mediated clearance for fibres is highly dependent on fibre length. Phagocytosis becomes increasingly difficult as fibre length increases, leading to the phenomenon of 'frustrated phagocytosis'. This phenomenon applies only to fibres. Short fibres ($<5 \mu\text{m}$) are cleared with relative ease, but as fibres become longer, and approach the size of AM diameter, they cannot be engulfed by a single macrophage (Davis, 1991). Macrophage motility is likely to be completely inhibited following the attempted phagocytosis of a long fibre. Therefore there will be negligible pulmonary clearance of fibres $> 16 \mu\text{m}$. There is ample experimental evidence for this (see paragraphs 3.15, 3.24 and 3.25).

5.9 When AMs fail to clear inhaled particles effectively, either because of excessive numbers (overload), or cytotoxicity (as with quartz) or frustrated phagocytosis (long fibres), this leads to a disruption of the normal homeostatic controls on the AM population; the activation of AMs caused by the continued presence of particles leads to the release of mediators which cause the influx of further AMs, monocytes and neutrophils to the sites of particle deposition, thereby enhancing the inflammatory response. Other secretory products released by activated AMs include growth factors which stimulate fibroblast activity. This might be viewed as a subversion of the normal physiological defense mechanisms which evolved for dealing with microbial pathogens.

5.10 Overall, it can be seen that the initial development of lung injury caused by inhaled particles is related to the ability of the AMs to effectively clear particles from the airway passages.

5.11 Because large quantities of short fibres are cleared with ease, this suggests that they are not directly cytotoxic to AMs. This is supported by experimental evidence with amosite, crocidolite and chrysotile. Donaldson *et al* (1993) suggest that direct toxicity to AMs does not play a predominant role in the mechanisms of fibre-induced lung injury. Rather, the ability of fibres to alter macrophage secretory activities is now considered to be important in the development of chronic lung disease.

Neutrophil involvement

5.12 Neutrophils are not normally present in the alveoli and their presence denotes an inflammatory reaction. Neutrophils have been implicated in the pathogenesis of many lung diseases through the release from activated cells of reactive oxygen intermediates and proteases (Macnee and Selby, 1993). The oxygen species released by activated neutrophils include superoxide anion, hydrogen peroxide, hydroxyl radicals, and hypohalous acids. A marked influx of neutrophils into alveoli has been observed in the acute inflammatory response to inhaled aramid fibres and chrysotile in rats (Warheit *et al*, 1992; Warheit, 1994; Rom *et al*, 1991). However, while the role of neutrophils in the acute phase of injury associated with short-term high level exposure to fibres appears to be well established, their role in the development of chronic disease, associated with prolonged low level inhalation of fibre may be relatively minor.

Mechanisms of injury to alveolar epithelium

5.13 As noted in paragraphs 5.9 and 5.12, the release of reactive oxygen species and proteases by activated neutrophils and AMs causes injury to alveolar epithelial cells. It is also possible that reactions catalysed on the fibre surface play a role in cytotoxicity. In this regard, the iron content of fibres is thought to be a factor in the redox activity of fibres. All types of asbestos have been shown to generate some active oxygen species in cell free systems via iron-catalysed reactions on the fibre surface. Crocidolite contains up to 36% iron, but chrysotile contains only about 2-3%. On a surface area basis crocidolite is more active in free radical generation than chrysotile. In studies cited by

Mossman (1993) it has been shown that the *in vitro* cytotoxicity of asbestos samples could be reduced by the addition of iron chelators such as desferrioxamine.

Interstitialisation

5.14 The ability of inhaled fibres to enter the interstitial tissues is thought to be important in relation to the potential to cause pulmonary fibrosis. The passage of chrysotile fibres from the airway spaces to pulmonary interstitium has been observed in rats (Brody *et al*, 1981). This passage appeared to involve the active uptake of chrysotile fibres by the epithelial cells. Subsequently, these fibres were phagocytosed by interstitial macrophages (IMs) and fibroblasts. Also, areas of airway epithelial damage may allow access of fibres into the pulmonary interstitium.

5.15 Evidence obtained by Adamson and Bowden (1990) and Adamson *et al* (1993) from studies involving IT administration of long and short crocidolite fibres in mice suggests that the intact alveolar epithelium may act as a barrier to the passage of fibroblast growth factors from activated AMs into the interstitium; it would appear that fibre penetration to the pulmonary interstitium is necessary in the development of pulmonary interstitial fibrosis. In addition, if the integrity of the epithelial barrier is compromised by direct or indirect fibre-induced injury, AM-derived growth factors may gain access to the pulmonary interstitium and stimulate fibroblast activity.

5.16 After reaching the pulmonary interstitium, fibre length and durability are important determinants of fibrogenic potential. Short fibres may be transported to local lymph nodes by IMs. In contrast, macrophage-mediated clearance of long fibres is ineffective, and allows the potential for repeated activation of IMs and a chronic inflammatory response. However, certain fibres may eventually dissolve or fragment, and the clearance of the resultant shortened fibre fragments will resume.

Fibre-induced macrophage responses

5.17 Exposure of macrophages to fibres has been shown to lead to the release of a number of secretory products. These cytokines act as potent chemoattractants for inflammatory cells, as well as fibroblasts, and act as growth factors which are involved in paracrine and/or autocrine regulation of cell proliferation. Two different kinds of cytokine growth factors are produced - competence and progression factors, which act at different stages of the cell cycle. Platelet derived growth factor (PDGF) and fibronectin are both competence factors for fibroblasts, and are required for these cells to move from the resting stage into G1 of the cell cycle (Sheppard and Harrison, 1992). PDGF has been shown to be released by AMs exposed to fibres both *in vivo* and *in vitro*. PDGF is also released from injured Type II alveolar epithelial cells. Macrophages obtained from the lungs of people with pulmonary fibrosis spontaneously secrete more fibronectin than macrophages derived from healthy people.

5.18 Insulin like growth factor (IGF-1) acts as a potent progression factor for fibroblasts, and stimulates competence-primed cells to complete G1 and move into the S phase of DNA synthesis. IGF-1 also stimulates collagen production by fibroblasts. AMs obtained from human lungs with asbestosis spontaneously secrete a fibroblast progression factor with IGF-1 like activity. At least six different AM-derived cytokines have been identified.

5.19 Lung fibroblast cells also secrete cytokines which are involved in feedback loops. For example fibroblasts can release fibronectin which may be involved in autocrine regulation of cell division (positive feedback); fibroblasts can also release prostaglandin E2 which inhibits fibroblast proliferation (negative feedback). Fibroblasts are therefore not simply target cells that respond to inflammatory mediators, but they are effector cells in their own right.

5.20 It would appear therefore, that the mechanism for the development of pulmonary fibrosis involves a disruption of the normal homeostatic regulation of fibroblast activities caused by the chronically enhanced secretion of cytokine growth factors, which in turn is provoked by the continuing presence of durable long fibres. However, the complex network of interactions between the relevant effector cells has not yet been precisely characterised.

Fibre-induced lung cancer

5.21 There is clear human and animal evidence for the induction of lung cancer by the asbestos group of mineral fibres, and there is also animal evidence for carcinogenicity with four different types of RCF (Mast *et al.*, 1995). There are differences in the chemical composition and mineralogical structure among the asbestos group of fibres, and there are physico-chemical differences among the RCFs. The common features shared by these substances are the possession of the long thin fibrous shape, and relative lack of solubility. There is no clear reason to suppose that there would be any significant differences in the mechanisms of lung cancer induction by these fibres.

5.22 Another property common to these fibres is that they have been noted to produce pulmonary interstitial fibrosis at an earlier stage to the onset of lung cancer. The pattern of evidence from sequential histological examinations in rats exposed to crocidolite, chrysotile and RCFs showed that early onset pulmonary fibrosis, that is within 3-6 months of exposure, appeared to act as a marker for a subsequent increase in the risk of lung cancer (McConnell *et al.*, 1994; Mast *et al.*, 1995). Davis and Cowie (1990) reviewed histological slides which had been archived from fibre carcinogenicity studies in rats, and noted that where tumours were found at an early stage of development, their origin from areas of fibrosis could be confirmed (see 4.32). There is also human evidence for an association between asbestosis and lung cancer (see 2.35-2.38). The animal evidence with RCFs suggests that exposures which are insufficient to cause pulmonary fibrosis will not lead to an increased risk of lung cancer. These observations are consistent with the view that fibre-induced lung cancer occurs via a threshold mechanism.

5.23 Tumours are usually thought of as developing either as a result of a genotoxic mechanism of action (for which it is not possible to identify a threshold), or via non-genotoxic mechanisms (for which it is often possible to identify a threshold). Electrophilic substances which are capable of covalently binding to DNA provide an example of a type of substance which would be regarded as genotoxic. It should be noted that genotoxicity does not necessarily imply cancer development, as it may result in cell death as an end-point (Mossman, 1994). A number of non-genotoxic mechanisms of carcinogenesis have been identified, including, for example, prolonged disturbance of endocrine function (Purchase, 1994). Another recognised mechanism involves cytotoxicity and increased rates of cell proliferation leading to cell transformation and malignancy i.e. mitogenesis leads to mutagenesis (Ames and Gold, 1990). This mechanism of carcinogenesis will eventually involve changes to DNA leading to the activation of oncogenes or damage to tumour suppressor genes.

5.24 There is ample evidence that increased rates of cell proliferation are a prominent feature of fibre toxicity. For example Type II alveolar cell hyperplasia was noted as part of the early inflammatory response to inhaled aramid fibres and chrysotile (Warheit, 1994). Alveolar bronchiolization (replacement of flattened alveolar epithelial cells with cuboidal ciliated cells) was seen within 3 months of exposure in rats exposed to crocidolite, chrysotile and RCFs (McConnell *et al.*, 1994; Mast *et al.*, 1995), and was a prominent feature of aramid fibre toxicity (Lee *et al.*, 1988). Evidence with aramid fibres suggests that cell proliferation may be reversible in the early stages following cessation of exposure (Warheit, 1994). However, fibrosis becomes irreversible with repeated exposures or with the persistent presence of durable fibres. The chronically

elevated levels of cytokines which stimulate cell proliferation are likely to increase the potential for cell transformation and carcinogenesis.

5.25 From these observations the following sequence of events can be postulated to account for the development of fibre-induced pulmonary fibrosis and lung cancer:

- (i) deposition of fibres in broncho-alveolar regions of lung;
- (ii) local tissue damage and inflammation/ influx of macrophages and neutrophils;
- (iii) proliferation of cells in vicinity of fibre deposition;
- (iv) with continued deposition these events are repeated leading to increased epithelial cell hyperplasia and thickening of alveolar walls with collagen deposition;
- (v) with time the fibrosis spreads to peri-bronchiolar regions;
- (vi) activated macrophages in the region of fibre deposition and tissue damage continue to release cytokine growth factors which maintain epithelial cell and fibroblast proliferation;
- (vii) enhanced rates of cell division increase the probability of errors in DNA replication in actively dividing cells leading to heritable mutations in daughter cells (most likely alveolar bronchiolar epithelial cells). In addition, release of active oxygen species from damaged cells and activated macrophages may add to DNA damage in surrounding cells (as may occur with prolonged tissue damage from whatever cause);
- (viii) further mutations may accumulate leading to metaplasia, neoplasia and eventually carcinoma development.

5.26 This sequence is supported by various lines of experimental evidence, and indicates that both the fibrosis and the lung cancer emanate from the same underlying chronic inflammatory condition. However, fibrosis is an earlier response and serves as a marker for a subsequent increase in the risk of lung cancer. According to this sequence, exposures which are insufficient to elicit chronic inflammation/cell proliferation (manifest, for example, as alveolar Type II cell hyperplasia or fibrosis) will not incur any increased risk of lung cancer, ie it is consistent with a threshold model for fibre-induced lung cancer.

5.27 An alternative hypothesis would be to invoke a direct genotoxic mechanism. Asbestos is not mutagenic in standard mutagenicity assays in bacterial and mammalian cells (Brown, 1990). There is evidence for genotoxicity with asbestos: Mossman (1994) lists a large number of *in vitro* studies in which evidence for chromosomal aberrations in mammalian cells was obtained with chrysotile and other fibres. However, this particular assay appears to be very limited in its usefulness for particulates. In one study, all particles tested ie chrysotile, a zeolite mineral and latex beads, produced increases in chromosomal aberrations in cultured human lymphocytes. The remaining studies were limited due to lack of appropriate positive or negative control particles. This suggests that no firm conclusions can be drawn from such *in vitro* studies with fibres.

5.28 Mossman (1994) discusses the available experimental evidence for fibre-induced genotoxicity and notes that the end-points measured in a number of studies (eg morphological transformation in rodent cells) are measures of cytotoxicity rather than genotoxicity. When considering the mechanism whereby fibres could lead to genotoxic changes two possibilities occur; one could be that when fibres become internalised by cells they may interfere with the correct functioning of the mitotic spindle, leading to malsegregation of chromosomes at cell division. The resulting level of chromosomal disruption is likely to be so gross that it would be incompatible with further cell growth (carcinogenic fibres are $>5 \mu\text{m}$ in length, which is relatively large compared to the size of a cell). The other possibility is that reactions catalysed at the

fibre surface may lead to the generation of free radicals which may damage DNA. Indeed, chrysotile has been shown to cause breakage and oxidative lesions in isolated DNA (Adachi *et al*, 1992). However, this observation is not supported by evidence of mutagenicity for asbestos in mammalian cell systems. Also, milling crocidolite asbestos reduces its carcinogenicity, but does not alter free radical production (Petruska, 1991). This suggests that free radical production cannot suffice as an explanation for the mechanism of fibre-induced carcinogenicity.

5.29 Overall, although there is some evidence from *in vitro* studies for fibres resulting in genetic damage, it is possible that these results reflect cytotoxicity rather than direct mutagenic events. There is a lack of convincing evidence for the ability of fibres to elicit direct genetic changes which could themselves lead on to carcinogenesis.

Fibre-induced mesothelioma

5.30 The precise method whereby inhaled fibres translocate to the mesothelium has not been defined, but inhaled fibres can deposit in the lungs in very close proximity to the sub-pleural basement membrane. One early study involving the short-term inhalation of radioactive asbestos in rats, found that over the course of some months there was a gradual accumulation of fibres towards the peripheral regions of the lungs, a phenomenon termed 'pleural drift' (see paragraph 3.6).

Mechanisms of pleural toxicity

5.31 In a recent study rats were given single IT doses of crocidolite, and the pleural leucocytes were investigated for up to 30 days post-exposure (Li *et al*, 1993). The results indicated that following the pulmonary deposition of crocidolite, there was some recruitment of pleural leucocytes, and changes in the *in vitro* production of interleukin-1 (IL-1) by these cells.

5.32 Increased DNA synthesis was measured in mesothelial and sub-pleural cells within a week of IT instillation of crocidolite in mice (Adamson *et al*, 1993). No fibres were found at the pleura at this time. Histological studies revealed that crocidolite fibres became incorporated into the underlying connective tissue of the bronchial and bronchiolar epithelium, and in some sections of the lung, peribronchial or peribronchiolar interstitium could be traced directly to the pleural surface with no intervening alveolar spaces.

5.33 After a brief inhalation exposure to chrysotile, substantial numbers of inhaled fibres were deposited within 1-2 mm of the visceral pleura in the rat within 29 days of exposure (Coin *et al*, 1992). Other work by these authors has shown that following a 5-hour inhalation exposure of mice to chrysotile, an enhanced uptake of tritiated thymidine by sub-pleural peripheral mesenchymal cells occurred within 24 hours of exposure.

5.34 These studies demonstrate that intra-pulmonary fibres can elicit pleural responses (recruitment/activation of pleural leucocytes, mesothelial cell proliferation) within hours or days of exposure. The contribution that such pleural responses might make towards the development of pleural disease is uncertain.

Current theories on the mechanism of mesothelioma development

5.35 Most of the information relevant to an understanding of the mechanisms of mesothelioma development comes from injection studies in rodents. Stanton and Wrench (1972) reported that the extent of pleural fibrosis roughly correlated with the incidence of mesotheliomas in IPL studies in rats. Since then Kane and McDonald (1993) have reported more detailed information on the morphological development of fibre-induced mesotheliomas in mice given weekly IP injections of crocidolite. The animals were given weekly IP injections rather than a single bolus dose

in an attempt to more closely model human exposure. The responses of the peritoneal mesothelium were followed in detail using a combination of stereomicroscopy and scanning electron microscopy (SEM). This author also found that the development of fibrosis of the peritoneal lining preceded the development of mesothelioma.

5.36 Based on the observations made by Kane a sequence of events has been proposed to account for the development of fibre-induced mesothelioma (McClellan *et al*, 1992; Rom *et al*, 1991). It can be judged from this sequence that the mechanisms involved are similar in principle to those involved in fibre-induced lung cancer, in that repeated cycles of cytotoxicity with regenerative hyperplasia are involved.

5.37 The sequence of events is as follows: fibres which reach the pleura or peritoneal lining are phagocytosed by mesothelial cells and macrophages. The release of active oxygen species by these cells causes acute injury to the mesothelial cell monolayer lining the pleural or peritoneal spaces. Further toxicity to the mesothelial cell layer is caused by active oxygen species generated by reactions catalysed on the fibre surface.

5.38 This acute injury to the mesothelial lining is repaired by proliferation of adjacent, uninjured cells. Growth factors released from macrophages following phagocytosis of asbestos fibres enhance mesothelial cell regeneration. Repeated episodes of mesothelial cell injury and regeneration may lead to the emergence of a subpopulation of autonomously proliferating cells. This preneoplastic cell population may accumulate additional genetic changes, resulting in the eventual formation of neoplastic mesothelial cell subpopulations.

5.39 Erionite is far more active than other fibres in producing mesothelioma, even when comparisons are made on similar numbers of similarly sized fibres (Coffin *et al*, 1992). Erionite fibres contain numerous internal channels which communicate with the surface of the fibre by minute pores (0.5-0.7 nm in diameter). This confers an enormous total surface area relative to other similarly sized fibres. These channels are thought to be the site of catalytic activity involving the formation of free radicals and active oxygen species. Although erionite does not contain iron it is believed that the coordination of endogenous iron to the surface of silicates via silanol groups can induce an electron exchange by the Fenton reaction, whereby the iron is reduced from the Fe^{3+} to Fe^{2+} with the release of hydroxyl radicals.

5.40 The evidence with erionite suggests that surface properties are important in fibre toxicity, and the ability to generate free radicals at the fibre surface may be particularly important in mesothelioma. Fibre durability also appears to be important for mesothelioma induction. IP studies have shown that soluble fibres of the dimensions normally associated with mesothelioma induction have failed to produce mesothelial tumours (see paragraph 4.9). Human evidence also shows that the risk of mesothelioma is greater with the more durable forms of asbestos (amphiboles) than with chrysotile, which undergoes more effective clearance from the lungs. From this, it would appear that the continued presence of fibres is necessary for development of mesothelioma. There is clear evidence that possession of the fibrous shape is also important (see Section 7). Stanton observed that short fibres ($<8 \mu\text{m}$) directly administered to the pleural cavity were cleared easily by pleural macrophages and transported to the local lymph nodes, whereas long fibres could not be cleared. Thus, it would appear that once long fibres gain access to the mesothelium, they will remain at this site for long periods, depending on their dissolution characteristics.

Summary of mechanisms of fibre toxicity

5.41 There has been considerable progress in recent years towards understanding the mechanisms of fibre toxicity and carcinogenicity. The development of pulmonary interstitial fibrosis is preceded by injury to the alveolar and bronchiolar epithelium. This results from direct toxicity caused by long fibres, but not short, and indirect toxicity

caused by the release of oxidants and enzymes from macrophages and neutrophils following the incomplete phagocytosis of long fibres. Cytotoxicity may also result from reactions catalysed at the fibre surface leading to the generation of oxygen-containing free radicals.

5.42 Airway epithelial damage may facilitate the passage of fibres from the airspaces into the pulmonary interstitium, which appears to be important in the development of pulmonary interstitial fibrosis. The mechanism for the development of pulmonary fibrosis is thought to result from the ability of fibres to provoke a chronic enhancement of the secretion of cytokine growth factors from effector cells. Some of the cytokine molecules thought to be involved in pulmonary interstitial fibrosis have been identified, but the complex network of interactions mediated by cytokines between the various effector cells has not yet been elucidated.

5.43 There is evidence for an association between fibre-induced pulmonary interstitial fibrosis and lung cancer, consistent with the view that the enhanced rates of cell proliferation associated with chronic inflammation and fibrogenesis predispose to neoplastic cell transformation. There is a lack of convincing evidence for the ability of fibres to elicit any direct genetic changes that might lead on to carcinogenesis.

5.44 The mechanisms of fibre induced mesothelioma are probably similar in principle to those for lung cancer, involving chronic inflammation and increased cell proliferation eventually leading to neoplastic transformation. However, there are still some uncertainties, for example, regarding the process and importance of fibre translocation to the mesothelial tissues.

5.45 Recent evidence shows that pleural changes (eg enhanced DNA synthesis in mesothelial cells) may be elicited by indirect effects not involving fibre penetration to the pleural membranes, but result from the deposition of fibres in the alveolar regions of the lung in close proximity to the sub-pleural membrane. The significance of this in relation to pleural toxicity is unclear.

Section 6

FIBRE TOXICITY TESTING STRATEGY

6.1 For scientifically based proposals for controls such as classification and labelling, and for risk assessment purposes, there is a need to be able to accurately predict or identify the health hazards of new and existing fibres. Advances in manufacturing technology, together with the declining use of asbestos, will lead to the use of new fibres for which there is a lack of an agreed, structured scientific framework by which their hazardous properties should be explored.

6.2 To address this problem there is a need to develop a rational fibre toxicity testing strategy. This requires a harmonised approach towards the acquisition and interpretation of all data perceived to be relevant to hazard identification. Achievement of this aim has been limited by the lack of agreed test method protocols.

6.3 Long term inhalation studies in laboratory animals have the potential to produce the most useful data for evaluating fibre toxicity and carcinogenicity. However, such studies are technically difficult, costly, time consuming, and do not always yield results which can be readily interpreted. Ideally, predictions about the health hazards of novel fibres would be made on the basis of the physical and chemical characteristics of the fibres, together with the results of a number of carefully selected short term *in vitro* and animal tests. Dose response characteristics could perhaps be predicted from mathematical modelling. However, mathematical modelling of fibre-induced lung injury and mesothelioma is still a very new field (Fran *et al*, 1994).

6.4 The successful design of a fibre toxicity testing strategy depends on a knowledge and understanding of the fundamental mechanisms of fibre toxicity, and on a knowledge of which physical and chemical properties of fibres are the most important determinants of toxicity.

6.5 The following is an outline proposal for a fibre toxicity testing strategy. The proposal is not meant to be rigidly prescriptive or definitive, but is presented as a starting point which may need to be modified in the light of test method development. It is not the intention to precisely specify the test methodology or to define the criteria for evaluation of results. However, an attempt is made to acknowledge some of technical aspects involved.

Stage 1 Physico-chemical evaluations and short-term toxicity tests

Solubility testing

6.6 Fibre durability is an important determinant of toxicity. The potential to cause chronic lung damage will be less with a relatively soluble fibre than with a more durable fibre, and thus indices of solubility in physiological solution would be a useful parameter in predicting hazard.

6.7 Factors to be addressed in evaluating fibre solubility include the effect of pH. It has been shown that glass fibre dissolution rates increase with increasing pH (Potter and Mattson, 1991). There are differences in the pH of intra- and extra-cellular fluids in the lungs and pleural tissues and thus indications of solubility at these pHs are required. Dissolution rates depend on the surface area exposed to the solution and therefore fibres of similar diameters should be used for meaningful comparisons of the dissolution of fibres of differing composition. The solubility behaviour of a fibre *in vitro* may not accurately reflect that *in vivo* due to deposition upon the fibre surface of various materials secreted by macrophages, and therefore the physiological solution chosen - simulated lung fluid for example, - and the flow-through conditions need to be considered in comparing results with different fibres.

6.8 Similarly, the measured end-points of fibre dissolution, either fibre weight loss or the dissolution fluid composition, need to be considered. Analysis of the dissolution fluids is difficult because some ions are already present in the physiological solution in concentrations far higher than those dissolved. Fibre weight loss measurements do not inform on whether selective leaching or network dissolution is occurring, nor do they address morphological changes such as the fragmentation of a fibre.

6.9 Furthermore, the test methods required for measuring fibre dissolution for mineral fibres may be inappropriate for organic fibres; in some cases incubations in physiological solutions with proteolytic enzymes may be more suitable.

Fibre reactivity

6.10 It has been shown that the cytotoxicity of asbestos samples is reduced when scavengers of active oxygen species are present and that this may be related to the ability to mobilise iron (Kane, 1993). Measures of the ability of fibres to generate oxygen containing free radicals in cell free systems, and when incubated with mammalian cells including macrophages would therefore provide a further useful indices of potential toxicity.

Short-term toxicity tests

6.11 As noted above, fibre durability is thought to be an important determinant of the potential to elicit chronic cell damage in the pulmonary and pleural tissues. However the influence of this particular parameter is unlikely to be discernible in short-term tests for toxicity. Nevertheless, although durability is important it is clearly not the only factor affecting cytotoxicity (see paragraph 3.30). Therefore, although short-term toxicity tests with fibres may have limitations, there is sufficient scientific justification to anticipate that meaningful results can be derived from well designed short-term tests.

6.12 One group of workers has been active in developing short-term toxicity *in vitro* and *in vivo* tests (Donaldson *et al*, 1988; 1993). For example, they reported upon the cellular inflammatory response obtained in mice following IP injection of mineral dusts. Parameters measured included total and differential cell count, and macrophage activation status of lavaged cells recovered at 2, 4 and 8 days. The response to titanium dioxide and coal dust was small, while aramid fibres, chrysotile and quartz induced a marked and sustained inflammatory response. This test appears to provide a rapid, simple and reliable *in vivo* test of the pathogenicity of particulate material.

6.13 The mechanisms of fibre-induced carcinogenicity would appear to involve repeated episodes of cytotoxicity followed by regenerative hyperplasia. Therefore measures of the ability of fibres to provoke increased cellular proliferation in target tissues (bronchiolar and alveolar epithelium, pulmonary interstitial fibroblasts, and cells in the region of the visceral pleura and sub-pleural tissues) would be useful. Cell proliferation assays would be ideally conducted *in vivo*, with the rat (and possibly the hamster as well) being the most appropriate species. Exposure to the fibres might be delivered by IT instillation as this would be technically easier and less costly than inhalation. However, short-term inhalation exposure (eg over 5 days) would be preferable. Fibres used should be selected to ensure that they are respirable. Uptake of tritiated thymidine or 5-bromo-2'-deoxyuridine in the target cells at intervals following a short-term inhalation or a IT exposure regime could be used to measure cell proliferation rates. The use of carefully selected positive control fibres (eg erionite, crocidolite) would be needed to aid in the interpretation of the results. The choice of what would constitute a suitable negative control group would need careful consideration. Cell proliferation could be measured in the target cells at weekly intervals post-exposure up to perhaps 6 weeks. Careful consideration would need to be given to the doses and method of delivery.

6.14 On the basis of the solubility assessments, fibre reactivity, and the results of perhaps three or more short-term tests such as those outlined above, conclusions could be drawn regarding the likely inhalation health hazards of the fibre and the need for further testing. A fibre which was judged to be highly soluble, of low surface reactivity, and of low biological activity in carefully chosen short-term toxicity tests might be judged to be of low concern, and may not require any further testing.

6.15 However, if any of the above criteria are not met, then the next stage in a toxicity testing strategy would be to conduct a joint lung clearance and histopathology study.

Stage 2 Joint lung clearance and histopathology

Lung clearance

6.16 Lung clearance of inhaled fibres is achieved by macrophage phagocytosis and removal via the mucociliary escalator, and this is more effective for short fibres. In the longer term, some fibres may undergo dissolution which may lead to a weakening of the fibre structure and eventual fragmentation, which will then facilitate clearance. The ability to induce chronic lung damage is likely to be related to lung clearance rates. A standardised method for measuring lung clearance is not available. Two examples of the methodology used are briefly described as follows: in a 2-year study reported by Bellman *et al* (1987), the persistence of a number of types of MMMF and asbestos was measured in rat lungs following IT instillation. Parameters measured included the numbers of fibres, diameters and length distributions of fibres retained in the lung ash, and leaching of various elements from fibres. Half-times of lung clearance were calculated for fibres <5 and >5 μm in length. In another study, Warheit *et al* (1992) investigated the lung clearance of inhaled aramid fibres in rats. In this study the lengths and diameters of retained fibres were measured over a 6-month period following a brief inhalation exposure. The fibres were recovered from the lungs following digestion in potassium hydroxide solution.

6.17 In the context of a fibre toxicity testing strategy it is essential to have a standard protocol for measuring lung clearance. Factors to be considered include the route of administration, dose, fibre size distribution of delivered dose, methods of recovering fibres from the lungs which are non-destructive to the fibres, methods for counting fibres (SEM or phase contrast optical microscopy (PCOM)), methods for measuring bivariate (lengths and diameters measured on the same fibres) fibre size distributions, and also the duration of the study. For relatively durable fibres, 6 months might be too short a time to allow a meaningful evaluation of lung clearance.

6.18 One proposal would be to perform the lung clearance study using IT instillation, as this would probably be technically simpler and cheaper to perform than inhalation exposure. Twice weekly instillations over 2 or 4 weeks of a standard number of fibres eg 1×10^9 (rather than a standardised gravimetric dose) with regular sacrifices up to 6 or 12 months post-exposure might be a reasonable approach.

6.19 Evidence for substantial lung clearance would suggest a relatively low degree of concern. Evidence for fibre durability, ie no significant changes in retained fibre lengths and diameters and little change in numbers of retained fibres over time, would raise concerns for the potential to cause long term damage. However, the criteria for evaluation, what is meant by 'substantial lung clearance' for example, would need to be defined.

Histopathology evaluations

6.20 Weighed lung tissue samples from the clearance studies could be taken for histopathological evaluation. This would allow some conclusions to be drawn about the inflammatory and fibrogenic potential of the instilled fibres. The absence of signs of

pulmonary interstitial fibrosis, chronic inflammation in the airway epithelial tissues, or pleural fibrosis at any sacrifice time would suggest an absence of hazard for inhaled fibres; and together with evidence for substantial lung clearance and *in-vitro* solubility would suggest that no further testing may be warranted. If only minor signs of inflammation were seen, and they were reversible within about 4 weeks of dosing, and clearance data showed some evidence for limited durability, then again low concern would be warranted, and no further testing could be justified. In contrast, evidence for pulmonary interstitial fibrosis would be of high concern, especially following a limited number of IT instillations.

Stage 3 Carcinogenicity testing

6.21 By this stage in the testing strategy, enough data should be available (*in vitro* solubility, fibre reactivity, inflammatory potential, lung clearance kinetics, cell proliferation activity, etc) to enable a decision as to whether further toxicity testing is warranted. If there are grounds for concern remaining at this stage then one option would be to conduct a full scale inhalation or IT carcinogenicity study. If this could not be justified (eg insufficient commercial use of fibre product) then another options would be to conduct a 6-month repeated dose IT study.

6.22 The justification for a 6-month IT study is that IT studies are less costly and less technically difficult than inhalation studies; limited evidence from IT studies suggests that such studies are more sensitive than inhalation studies; evidence from inhalation studies with RCFs and with chrysotile shows that the early onset (3-6 months) of irreversible pulmonary fibrosis appears to act as a marker for the subsequent development of lung cancer. Therefore, with once or twice weekly instillations of fibres over 6-months it should be possible to make a worthwhile investigation of the potential to cause pulmonary and pleural toxicity. Evidence for the development of fibrosis in either of these sites would be at least suggestive of the potential for carcinogenicity. Therefore, a fibre might be presumed to be carcinogenic on the basis of clear evidence of irreversible fibrosis in pulmonary or pleural tissues in a 6-month study of this nature. Criteria for the severity of the fibrosis which would warrant this conclusion would need to be developed. On the other hand, if only minor pulmonary or pleural changes were seen, then the potential for carcinogenesis with more prolonged exposures might be viewed as negligible. Careful consideration of the dose levels and the use of positive and negative control groups would be needed in such a study.

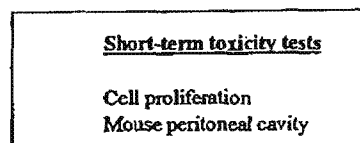
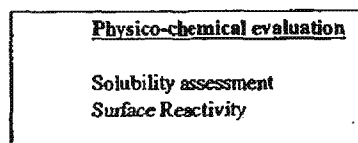
6.23 The alternative to performing a 6-month IT study to predict carcinogenic hazard would be to conduct a life-time inhalation or IT carcinogenicity study. There are advantages and disadvantages in either case as discussed in the section on Animal Studies. Only an inhalation study would allow the investigation of dose-response characteristics with the possibility for identifying NOAELs. Dose-response relationships in long-term IT studies appear to be highly erratic (Coffin *et al.* 1992), which is presumably due to the uneven fibre deposition patterns which may occur with IT instillation. Thus, a long-term IT study would be limited to an assessment of carcinogenic hazard only.

Summary of fibre toxicity testing strategy

6.24 The successful design of a fibre toxicity testing strategy requires an understanding of the mechanisms of fibre toxicity, and also of which physico-chemical properties most closely relate to hazard. From the progress which has been made in the field of fibre toxicology in recent years, a testing strategy can be proposed which should provide a rational basis for hazard assessment (see schema for proposed testing strategy overleaf).

FIBRE TOXICITY TESTING STRATEGY

STAGE 1



Evaluation of Results

Conclusions

Low Concern

Grounds for concern

No further testing

Proceed to Stage 2

STAGE 2

6-month Joint Lung Clearance and Histopathology Study

Evaluation of Results

Evidence for effective lung clearance
Evidence for lack of histopathological changes

Evidence for durability
Evidence for significant histopathological change

No further testing

Proceed to Stage 3

STAGE 3

Potential for substantial human exposure

Lifetime inhalation carcinogenicity study
OR
Lifetime IT carcinogenicity study

Limited human exposure

6-month repeated dose IT study

If irreversible pulmonary or pleural fibrosis results then regard as potentially carcinogenic. Fibre must be regarded as carcinogenic unless counteracted by results from a lifetime carcinogenicity study.

Section 7

RELATIONSHIP BETWEEN FIBRE SIZE AND TOXICITY

7.1 The aim of this section is to briefly summarise the toxicological evidence relating to the importance of fibre size and toxicity. As described in paragraph 1.1, the definition of a regulated fibre as used for counting purposes according to the UK Health and Safety Executive is that of a particle of length $> 5 \mu\text{m}$, and diameter $< 3 \mu\text{m}$, and with an aspect ratio (length to diameter) of $> 3:1$ as measured by PCOM using the membrane filter method. This definition was introduced in the UK in the 1960s (BOHS, 1968). The fibre count obtained using this method will not take account of fibres finer than $0.25 \mu\text{m}$, which cannot be seen by light microscopy.

7.2 The historical background leading to the definition, together with the available supporting scientific evidence were reviewed by Walton (1982). Walton concluded that 'considerations of respirability and biologic activity together suggest that the counts should be of fibres in the size range: length $> 5\text{--}10 \mu\text{m}$ but less than $100 \mu\text{m}$; width $< 1.5\text{--}2 \mu\text{m}$ (no minimum specified); aspect ratio $> 5:1$ to $10:1$ '. The suggestion to increase the aspect ratio was made in order to reduce the number of non-asbestiform particles which would be counted with the existing definition.

7.3 There are a number of fibre properties which are likely to exert an influence on toxicity - chemical composition, surface properties and durability for example. However, it is the presence of the long thin fibrous shape which appears to be the most important common factor necessary to produce the full spectrum of fibre-related toxicity. The influence of fibre size can be most clearly appreciated in relation to the efficiency of deposition in the lung; the subsequent clearance rates from the lungs; and also the mechanisms of toxicity at a cellular level.

Influence of fibre size on the efficiency of deposition in the lungs

7.4 The regional deposition efficiency of inhaled fibres is mainly a function of fibre diameter, and also density, with fibre length and aspect ratio being of lesser importance. The long thin shape of fibres allows inhaled fibres to move in parallel with the moving airstream, and deposition is more likely to occur at sites of branching within the respiratory tract, where flow becomes more turbulent. Thus relatively little deposition of inhaled fibres is anticipated in the trachea and main bronchi. Maximum alveolar deposition efficiency of inhaled fibres in humans is expected to occur with fibres of actual diameters about $1 \mu\text{m}$, and to tail off with values above this; so that mineral fibres with diameters $> 3 \mu\text{m}$ would be essentially non-respirable. For organic fibres with a lower density, values slightly greater than this may apply. With increasing fibre length, there is a greater probability of deposition due to interception in the upper airways, but nevertheless fibres of lengths $> 200 \mu\text{m}$ have been found in the alveolar region of the human lung (Craighead, 1982).

7.5 As described in paragraph 3.5, the pulmonary deposition of a range of fibres types (chrysotile, crocidolite, silicon carbide whiskers and aramid fibres) has been investigated in inhalation studies in rats. These studies consistently show that inhaled fibres deposit preferentially around the junctions of the terminal bronchioles and alveolar ducts, which are also the initial sites of development of asbestosis. Histological evidence from animal studies shows that with time, the fibrotic process progresses, and eventually both parenchymal and peribronchiolar fibrosis is also seen. It appears therefore that the disease may spread to areas away from the initial sites of fibre deposition.

7.6 In a recent study, cited in a review by Lehnert and Oberdorster (1993) the effects of inhaled chrysotile in rats were investigated by measuring the incorporation of tritiated

thymidine in cells of the larger and smaller conducting airways. No increases in cell labelling were seen in tracheal epithelium up to 33 hours post-exposure, but there were marked increases in the more distal regions of the lung, with the largest increases found in the terminal bronchiolar regions. These findings are consistent with the view that inhaled fibres deposit preferentially in the terminal bronchiolar regions of the lung, but may also indicate a lack of susceptibility of the larger conducting airways to fibre-induced cytotoxicity.

Influence of fibre size on lung clearance rates

7.7 The potential for disease development is a function of the persistence of fibres deposited in the lungs. Persistence in the alveolar regions of the lungs is determined by two distinct processes - macrophage-mediated clearance, and chemical dissolution. With respect to dissolution, chemical composition is probably the major determinant. However, fibre size plays a critical role in determining macrophage clearance rates (Davis, 1991). Clearance by macrophages is only effective for fibres which are short enough to be fully engulfed by individual phagocytes, and becomes increasingly less effective with increasing fibre length.

7.8 From evidence presented in paragraphs 3.24 and 3.25 it is clear that macrophage clearance is less effective for 10 μm length fibres than for 5 μm , and is ineffective for fibres of 16 μm and longer.

Influence of fibre size in relation to cellular mechanisms of toxicity

7.9 As discussed by Davis (1991) short-fibres ($<5 \mu\text{m}$) can be cleared from the lungs by alveolar macrophages with relative ease. However, the attempted phagocytosis of long fibres impairs macrophage motility, leading to the release of inflammatory mediators and growth factors which can culminate in fibrosis (see paragraphs 5.8 and 5.13). It is also assumed that the partial phagocytosis of long fibres allows the escape of lysosomal enzymes into the extra-cellular medium, and that these enzymes may contribute substantially towards fibre-induced toxicity (Brown *et al*, 1990).

Human evidence

7.10 There is little information to be gained on the importance of fibre size from human studies. One limitation is that in the workplace, workers have been exposed to a range of fibre sizes, and thus it is difficult to attribute the observed health effects to a particular size category. Often, exposure data in epidemiological studies are limited, and provide little information on fibre size distributions. Autopsy studies have provided some information on the lung fibre size distributions in relation to disease. However such information must be interpreted with caution. For example, the development of asbestosis is likely to impair clearance rates so that the presence of large quantities of short fibres in the lungs at autopsy cannot be taken as evidence that such fibres were causal to the initial disease development.

7.11 The lung cancers associated with asbestos have been described as being typically bronchogenic in origin, although more peripherally situated lung cancers have been observed in some series (Doll and Peto, 1985; Rosenthal, 1993). For example, in a study cited by Rosenthal (1993), 7 of 13 lung cancers found in textile workers were 'situated in the periphery of the lung, usually near the base'. Similarly, in a further study, of 88 autopsied lungs with carcinoma associated with asbestos, 41% were found to occur at 'peripheral sites'. In addition, Rosenthal notes that the tumours seen at autopsy are often in advanced states, and have obliterated their point of origin.

7.12 The influence of cigarette smoking may be relevant to this discussion. David and Donaldson (1993) were of the opinion that the lung tumours caused by asbestos in cigarette smokers are most often bronchial carcinomas, but in non-smokers they are

more peripherally situated. However, these authors consider that it is difficult to be definitive on this subject as there are so few cases of lung cancer attributable to asbestos in non-smokers.

7.13 The bronchial location of asbestos-associated lung cancers in humans raises the question that perhaps fibres which penetrate the epithelium of the larger conducting airways, either by direct deposition following inhalation exposure, or via mucociliary clearance of respired fibres, may be implicated in the development of lung carcinoma. This consideration appears to be of particular relevance to smokers where the efficiency of the mucociliary escalator may be impaired.

7.14 However, little deposition of inhaled fibres upon the epithelium of the larger conducting airways would be anticipated as discussed in paragraphs 7.4 -7.6. Also, the bronchial epithelium is covered by a blanket of mucus which is continually wafted towards the throat for swallowing or expectoration. An intact mucus layer will prevent direct contact of deposited fibres with epithelial cells, and the mechanical action of the muco-ciliary escalator should ensure that the residence time of fibres depositing in the bronchial passages is short. These considerations suggest that fibres depositing in the larger conducting airways are unlikely to be involved in the initiation of disease development.

7.15 It has been noted in a detailed review of the asbestos-related diseases in humans that in cases of asbestosis 'the bronchi are normal unless the patient has been a heavy smoker...' (Craighead *et al*, 1982). Animal evidence indicates that tissue damage and fibrosis are initiated at the favoured sites of fibre deposition, the broncho-alveolar regions, but that with time, the fibrotic process may progress and spread to peri-bronchiolar sites.

7.16 Walton (1982) presented data from studies in which detailed transmission electron microscopy measurements of the fibre size distributions in the air of the workplace in mining and bagging operations for crocidolite, amosite and chrysotile were made at locations in South Africa and Canada. The bagging operation data are useful because this is the material which was delivered to asbestos-product manufacturers. With respect to fibre diameter values in bagging, the results showed that the median diameter for crocidolite was 0.086 μm (range 0.011 -0.84 μm); amosite had a median diameter of 0.26 μm (range 0.021-2.14 μm); the corresponding figures for chrysotile were 0.059 μm (range 0.013-2.29 μm).

7.17 Clearly, the majority of airborne asbestos fibres found in these surveys had diameters which were in the sub-micron size-range, and these diameters were unlikely to increase with further processing in asbestos-user industries. Information is also presented by Walton (1982) in relation to the dimensions of fibres found in the human lungs post-mortem showing that the majority of lung fibres had diameters in the sub-micron size range. Walton notes that in general, airborne asbestos fibres of diameter >1.5 μm are rare, so that few are to be expected in the lung. Although these considerations are not able to demonstrate that thick fibres (>3 μm) are not hazardous, they suggest an association between asbestos fibres with diameters in the sub-micron size range and the development of lung cancer.

7.18 Just as there is a lack of definitive evidence on the role of fibre diameter from human studies, there is little information on fibre length. However, the slopes of the dose-response curves for lung cancer associated with chrysotile asbestos are much steeper in the textile industry than in chrysotile mining and milling (Doll and Peto, 1985; Hughes and Weill, 1986). This may be because the chrysotile used in the textile industry was derived from the longer silkier grades, and led to a higher proportion of long fibres (>15 μm) in the air of the workplace than would be found in mining and milling. Thus, comparison of the lung cancer rates induced by chrysotile in these different industries would suggest that fibre length is important.

Animal evidence

7.19 Much of the available knowledge relating to the importance of fibre size in disease development comes from animal studies, chiefly in rats. The experimental methods used include inhalation and IT exposure, which provide information on the biological responses of the pulmonary and mesothelial tissues, and IPL and IP methods which provide information on the response of the mesothelium only.

7.20 The main difficulties in conducting experiments to investigate the role of fibre size relate to the expense and technical expertise required to produce sufficient quantities of size-selected fibres, particularly in the amounts needed for inhalation work. Interpretation of most of the early reports on inhalation studies in animals has been limited by inadequate information on exposure conditions, in terms of fibre numbers, fibre size-distributions, and the concentrations of non-fibrous dust.

Evidence from intra-pleural administration studies

7.21 The experiments of Stanton and Wrench (1972) involving IPL implantation of fibrous glass, asbestos, non-crystalline silica and metal fragments into rats, indicated that carcinogenic potential was related to the structural shapes of these materials, rather than to physicochemical properties (see paragraphs 4.7 and 4.8). An analysis was undertaken of the fibre size distributions of the 17 different samples used. Two types of fibrous glass with mean diameters ranging from 5-10 μm produced 4 mesotheliomas in 91 rats. Two other types of especially fine glass fibre, 0.06-3 μm in diameter produced mesotheliomas in 12-18% of treated animals. The results of this study therefore indicated that the potential to induce mesothelioma may be related to fibre diameter.

7.22 Further work using the same methodology was undertaken with 16 different fibrous glass samples, of a range of chemical compositions and dimensions (Stanton *et al*, 1977). Equal weights (40 mg) of the glass fibres were administered to groups of 30 rats and the biological responses of the pleural mesothelium were noted subsequently. Analyses of the tumour rates in relation to the dimensions of the glass fibres indicated a high probability of tumour induction with fibres of lengths $>8 \mu\text{m}$, and diameters $<1.5 \mu\text{m}$. However, equal weights of fibre samples were administered, and therefore the fine glass fibre samples contained a far larger total number of fibres than the coarser samples. This may also have contributed to the increased tumour incidences with the finer glass samples.

7.23 Stanton *et al* (1981) reported further studies with asbestos samples and with a number of glass fibre samples employing the same methodology as above. Analyses of the tumour rates in relation to fibre dimensions indicated that the probability of tumour induction was highest for fibres of diameter $<0.25 \mu\text{m}$ and length $>8 \mu\text{m}$ (fibres with these size characteristics have become known as 'Stanton fibres'). However, a good correlation of tumour rates with fibres of $<1.5 \mu\text{m}$ diameter and $>4 \mu\text{m}$ in length was also found.

Studies employing intra-peritoneal injection

7.24 As described in paragraph 4.9 a long series of IP studies in rats began in the 1970s conducted by Pott and his colleagues (Pott, 1993). Based on the results of these studies Pott has been able to formulate a hypothesis to describe the relationship between fibre size and carcinogenicity. The hypothesis is consistent with the findings of Stanton *et al*, in suggesting that the highest potential for carcinogenesis resides with long thin fibres, although it gives no sharp cut off values for fibre length, nor does it suggest a minimum diameter value below which there would be a lack of carcinogenic potential. Thus even short fibres ($<5 \mu\text{m}$ in length) may have some carcinogenic potential. In addition, the

hypothesis of Pott requires that fibres should be sufficiently durable. Thus, gypsum fibres of dimensions generally considered to be carcinogenic did not produce a positive result in the IP test, presumably due to a lack of durability.

7.25 Although the use of IP testing for fibrous substances is perhaps most closely associated with Pott and his colleagues, studies employing this method have been reported by other workers. Davis *et al* (1986) injected samples of short and long-fibre amosite into the peritoneal cavity of rats. Both samples had been prepared from the same batch of South African amosite, the short fibre sample being prepared by milling and water sedimentation. The two samples did not differ substantially in relation to fibre diameter, but the length characteristics were significantly different. The short-fibre sample contained only 1% of fibres $>5 \mu\text{m}$ in length, whereas the long fibre sample contained 30% $>5 \mu\text{m}$, and 11% $>10 \mu\text{m}$. The long fibre amosite produced mesotheliomas in 95% of treated rats, with a mean induction time of about 500 days. The short fibre samples produced only 1 mesothelioma after 837 days among 24 rats. Comparison with an earlier study by the same group of workers using UICC amosite which has fibre lengths intermediate between the long and short amosite, showed that it produced the same proportion of mesotheliomas as the long amosite sample at the same dose level.

7.26 There are a number of possible explanations for the development of the one mesothelioma with the short-fibre sample of amosite. It may have been caused by the very few long fibres which may have been present, or it may be that very large numbers of short fibres if presented to the target tissue, can lead to mesothelioma. Overall, it can be reasonably concluded that there is either an absence of hazard, or a very low hazard in terms of ability to cause mesothelioma with short ($<5 \mu\text{m}$) fibres. However, these results demonstrate that fibres of intermediate lengths (8-10 μm) can cause mesothelioma. These studies do not allow any specific conclusions to be reached concerning the importance of fibre diameter.

7.27 Similar IP studies were conducted at a later date within the same laboratory using samples of long and short chrysotile (Davis and Jones, 1988). Due to technical difficulties it was not possible to produce a chrysotile sample which was entirely free of the presence of long fibres (see Table 3 for fibre size data) and both fibre samples produced mesotheliomas in over 90% of rats at the top dose of 25 mg. At 2.5 mg the incidences of mesothelioma in rats exposed to the long and short fibre samples were 90% and 33% respectively. At 0.25 mg, 67% of rats exposed to the long fibre sample developed mesothelioma, whereas no such tumours developed at this dose level in rats exposed to the short fibre sample.

Evidence from animal studies employing intra-tracheal administration

7.28 As noted in paragraph 4.3, the work of Vorwald *et al* (1951) showed that finely ground samples of asbestos did not cause pulmonary fibrosis in laboratory animals when instilled into the trachea, whereas smaller total amounts which contained long fibres were highly fibrogenic.

7.29 IT studies conducted with long (mean length 24.4 μm) and short (mean length 0.6 μm) crocidolite in mice showed marked differences in the biological responses between these fibres as described in paragraphs 5.17-5.19 (Adamson and Bowden, 1990 and Adamson *et al*, 1993).

Evidence from animal inhalation experiments

7.30 Most inhalation studies have involved exposing animals to aerosols which are polydisperse in terms of fibre size distributions, and thus it is difficult to draw any firm conclusions about the relative toxicity of different size fibres.

7.31 The most convincing evidence available from inhalation experiments on the relationship between fibre size and toxicity, has been produced by Davis *et al* (1986) using the samples of short and long fibre amosite described in paragraph 7.17. Groups of 48 rats were exposed to 10 mg.m^{-3} of these asbestos samples for 12 months, and were then maintained for the remainder of their lifespan. In rats exposed to the short-fibre samples no pulmonary fibrosis or neoplasms developed. However, in rats exposed to the long-fibre sample there was extensive fibrosis, and a 30% incidence in tumours (three adenomas and eight carcinomas in the lungs, and two pleural mesotheliomas). Comparison with earlier inhalation experiments conducted in the same laboratory with UICC amosite revealed that similar exposures had produced very little fibrosis, and only two benign adenomas in 40 rats. Combined with the evidence from the IP studies with the same asbestos samples, these observations suggest that fibres $<5 \mu\text{m}$ in length are of low concern for disease development at any site; fibres in the region of $8\text{--}10 \mu\text{m}$ can cause mesothelioma; but longer fibres, perhaps in the region of $15\text{--}25 \mu\text{m}$ are needed to cause pulmonary fibrosis and lung cancer.

7.32 Subsequently, long-term inhalation studies in rats exposed to equal mass concentrations (10 mg.m^{-3} of respirable dust) of long or short chrysotile were conducted in the same laboratory (Davis and Jones, 1988). Due to technical difficulties it was not possible to obtain a preparation which was entirely free of long fibres (see Table 3 for details of fibre sizes). Rats from both groups developed pulmonary fibrosis, malignant lung tumours, and mesotheliomas although the numbers were higher in rats exposed to the long fibre sample.

7.33 Further evidence for the importance of fibre length in the development of mesothelioma comes from inhalation studies reported by Wagner described by the IPCS (1986). The incidence of mesotheliomas was 96% in rats exposed to erionite fibres by inhalation (37% of fibres were $>5 \mu\text{m}$ in length). When short fibre preparations of erionite were used under similar exposure conditions (only 2% of the fibres were $>4 \mu\text{m}$ in length) there were no mesotheliomas.

Evidence from studies in vitro

7.34 The toxicity of fibres as expressed in *in vitro* systems is not dependent upon the normal physiological processes of deposition and clearance. Hence, the intrinsic pathogenic properties of different fibre types can be directly compared. The evidence from such studies clearly shows that long fibres lead to more cytotoxicity than short fibres of the same mineral composition, although there is no clear pattern of evidence in relation to fibre diameter (Petruska *et al*, 1991; McClellan *et al*, 1992).

7.35 The detachment of cultured human epithelial cells from their substrate *in vitro* was caused by long amosite fibres, but to a much smaller extent with short amosite fibres of the same diameter (Donaldson *et al* 1993). This *in vitro* evidence indicates that perhaps long fibres but not short, can directly damage (cause loss or detachment) of epithelial cells. It is possible that epithelial cell damage occurring in the broncho-alveolar regions of the lung enhances the potential for fibres to penetrate into the interstitium, which is thought to be important in relation to the development of pulmonary fibrosis.

7.36 The results of experiments described in a review by McClellan *et al* (1992), demonstrate an association between fibre length and toxicity in cultured mammalian cells exposed to refractory ceramic fibres. Four compositions of size-selected fibres were compared (mean lengths for the samples were 9, 17, 22 and $24 \mu\text{m}$). In both cell proliferation and genotoxicity assays, the longer fibres were significantly more active than the shorter fibres.

7.37 Evidence relating to the importance of fibre diameter from *in vitro* studies is less clear. In a cell transformation assay in cultured Syrian hamster embryo cells it was found that thin glass fibres (average diameter 0.13 μm) produced higher cell transformation rates and were more cytotoxic than thicker fibres (average diameter 0.8 μm) when exposure was compared in terms of fibre mass (Hesterberg and Barrett, 1984). However, when exposure was compared in terms of fibre number the thicker glass fibre induced more transformed colonies per fibre. Overall, it seems that no firm conclusions can be drawn regarding the importance of fibre diameter based on the limited evidence available from *in vitro* studies.

Summary of the relationship between fibre size and toxicity

7.38 There is good evidence that longer fibres are more toxic than equal masses of shorter fibres of the same composition. Results from IP and inhalation studies in rats with long and short fibre amosite suggests that short fibres (<5 μm) pose little if any concern for disease development at any site, fibres of lengths at least in the region of 10-15 μm are necessary to produce disease in the pulmonary parenchyma, but shorter fibres in the region of 8-10 μm can cause mesothelioma. There would be no biological reason to suppose that a sharp cut-off value in fibre length would exist to separate hazardous from non-hazardous fibres. This suggests that there would be no justification for increasing the current value of 5 μm as the length of a 'regulated' fibre.

7.39 There is little firm evidence available on the role of fibre diameter. Finer fibres may appear to be more toxic simply due to their greater efficiency of lung deposition following inhalation exposure. There is no evidence that thinner fibres are more toxic than thicker fibres at a cellular level, when comparisons are based on numbers of fibres. Overall, based on considerations of patterns of regional deposition in the respiratory tract in relation to disease development, it is concluded that the focus of concern for counting purposes should continue to be with those fibres which are deemed to be respirable ie capable of depositing within the broncho-alveolar regions of the lung. For mineral fibres this would include all fibres <3 μm in diameter. However, for organic fibres of a lower density, which may possibly display more efficient lung penetration and deposition, a slightly higher value for fibre diameter should be considered. There are no toxicological reasons to suggest that the minimum diameter for a regulated fibres should be reduced below the current value. There is a lack of specific toxicological evidence relating to aspect ratio.

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ABBREVIATIONS

IPL	intrapleural
IP	intraperitoneal
IT	intratracheal
WHO	World Health Organisation
MMMF	man-made mineral fibre
MMUF	man-made vitreous fibre
RCF	refractory ceramic fibre
PCOM	phase contrast optical microscopy
mpcf.y	millions of particles per cubic foot.years
SMR	standardised mortality ratio
CMD	count median diameter
CML	count median length
AM	alveolar macrophage
MEL	maximum exposure limit
GI	gastrointestinal tract
IARC	International Agency for Research on Cancer
UICC	Union Internationale Centre le Cancer
NOAEL	No observed adverse effect level
SPF	specific pathogen free
PDGF	platelet derived growth factor
SEM	scanning electron microscopy

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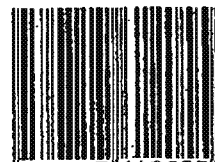
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