4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

4.1 Deposition, retention and clearance

The deposition of airborne fibrous and non-fibrous particles is defined as the active loss of these particles from the air during respiration, as a result of inelastic encounter of the airborne particles with the respiratory epithelium. Clearance from the site of deposition pertains to the removal of these deposited particles by various processes over time, whereas retention is the temporal persistence of particles within the respiratory system (Morrow, 1984). Thus, the amount retained (R) is defined by the amount deposited (D) minus the amount cleared (C) ($R = D - C$).

The deposition of inhaled fibres in the respiratory tract is a function of their physical characteristics (size, shape and density) and of the anatomical and physiological parameters of the upper and lower airways. Chemical composition has no role in deposition of airborne fibres in the respiratory tract, and, therefore, there is no need to differentiate between fibre types when discussing deposition phenomena. The clearance of deposited fibres from the respiratory tract, however, is dependent on both physical and chemical characteristics of fibres, and therefore the clearance and resulting retention behaviour within the respiratory system can vary widely between different fibre types; the different fibres deposited in the lung are subject to the same clearance processes, which together determine the biopersistence of the fibres. Thus, since a general understanding of deposition, retention and clearance is necessary to appreciate the importance of biopersistence, these topics are discussed briefly below for all fibres together rather than considering specific categories of vitreous fibres separately.

The main processes leading to the deposition of inhaled fibres operate throughout the respiratory tract, whereas the mechanisms that clear deposited fibres from different regions of the respiratory system vary considerably and, therefore, contribute to a different degree to the retained fibre burden at sensitive sites in the lung. When discussing deposition and retention it is, therefore, convenient to divide the respiratory system into three compartments as follows: the extrathoracic region (ET, consisting of anterior and posterior nose, larynx, pharynx and mouth); the tracheobronchial region (TB, consisting of trachea, bronchi and bronchioles down to the terminal bronchioles) and the alveolar–interstitial region (AI, including respiratory bronchioles, alveolar ducts and sacs with alveoli and pulmonary interstitium). A somewhat more detailed classification in which the respiratory system was divided into four compartments was

presented by the International Committee on Radiological Protection (ICRP) Task Group (ICRP, 1994). In this scheme the TB region was separated into large bronchi (BB) and small bronchioli (bb) compartments. Since the main clearance process in these two regions is the same (ciliary movement) and differs only in rate, the TB region is considered as one compartment here. The following sections summarize those concepts of deposition, clearance and retention that are known for both spherical and fibrous particles and that are helpful for the evaluation of fibre toxicity and carcinogenicity. Such an evaluation will, to a large degree, be based on a definition of fibre biopersistence as discussed in section 4.1.3.

4.1.1 *Deposition*

Several publications have described in detail the deposition behaviour of inhaled particles throughout the respiratory tract in humans and rats in general, as well as pointing out specific differences that pertain to fibres (Heyder, 1982; Morrow & Yu, 1985; Stöber *et al*., 1993; ICRP, 1994; Schlesinger *et al*., 1997; Asgharian & Anjilvel, 1998; Asgharian & Yu, 1988, 1989; Dai & Yu, 1998). Some of the main points are summarized below. Airborne particles and fibres are often described as being respirable or inhalable. The terms 'respirability' and 'inhalability' have very different meanings (defined by the American Conference of Governmental Industrial Hygienists (ACGIH), 2001).

Inhalability refers to the ratio of the particle (fibre) concentration in the inhaled air to that in the ambient air. Inhalability decreases with increasing particle (fibre) size due to increasingly higher settling velocities in air and inertia for increasing particle sizes (> 5–10 μm) before entering the airways; it is also dependent on wind velocity.

Respirability is the ratio of the concentration of airborne particles (fibres) penetrating to the alveolar region of the lung to that in the ambient air. Respirability generally increases with decreasing size of the particles (fibres), unless they become extremely small (i.e. fibrils, of nanometer size).

Using these definitions, the European Committee of Standardization (CEN, 1993) and ACGIH (2001) defined an inhaled particulate mass for those materials that are hazardous when deposited anywhere in the respiratory tract; a respirable particulate mass for those materials that are hazardous when deposited in the alveolar region; and a thoracic particulate mass for those materials that are hazardous when deposited anywhere in the lower respiratory tract (TB and AI regions). Thus, restricting the evaluation of fibres to 'respirable' fibres would be to ignore those fibres depositing in the TB region of the respiratory tract. The TB region is important when considering the pathogenicity of fibres in humans, since it is known from research on workers exposed to asbestos that this region is a potential target area for adverse health effects induced by fibres, in particular, chronic inflammation and bronchogenic carcinoma (Churg & Green, 1998). Acute nasal effects induced by fibres have also been

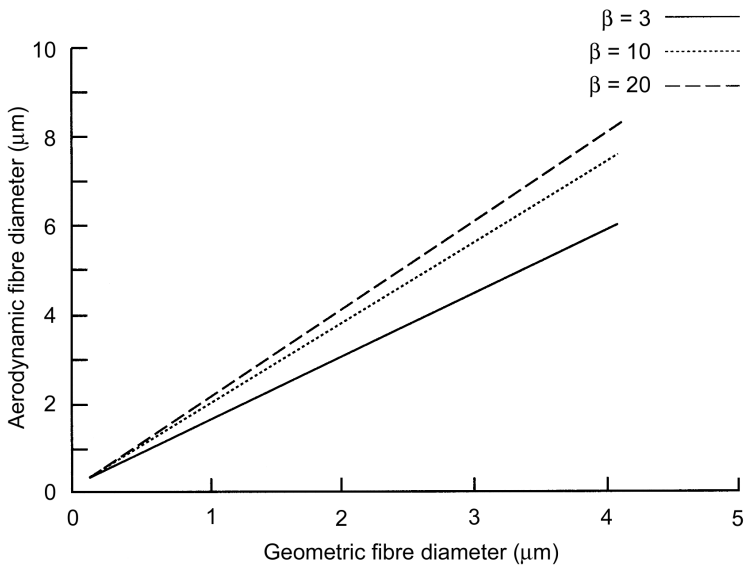
observed; therefore, the inhalable fibre fraction must be considered as well (INSERM, 1999; see also section 4.2).

Two important parameters that affect the deposition of airborne particles (fibres) are their aerodynamic and thermodynamic properties. The equivalent aerodynamic diameter is defined as the diameter of a spherical particle of unit density which has the same terminal settling velocity in still air as the particle (fibre) in question. Deposition due to aerodynamic behaviour becomes less important for particles with sizes < 1 μm. Below a particle size of 0.5 μm, thermodynamic properties prevail, and deposition of these particles is governed mainly by the diffusional movement induced by Brownian motion of gas molecules.

For a spherical particle, the geometric diameter multiplied by the square root of the specific density of the material gives the aerodynamic diameter. For a non-spherical particle, a shape factor also needs to be considered; although, for fibres in particular, their aerodynamic diameter is mostly governed by their geometric diameter, their elongated shape (fibre length) and their specific density. Figure 9 illustrates this for fibres of different aspect ratios (length:diameter) and with a density of 2.7 g/cm^3 (see Oberdörster, 1996).

The main mechanisms by which inhaled fibres deposit in the respiratory tract are impaction, sedimentation, diffusion and interception (Asgharian & Yu, 1988, 1989; Dai & Yu, 1998). Electric charges on fibres can also significantly enhance deposition due to

Figure 9. Correlation between aerodynamic and geometric diameter of fibres of different aspect ratios (β)



From Oberdörster (1996)
Specific density of fibre material is 2.7 g/cm^3.

generation of image charges in the airway walls; however, in contrast to asbestos fibres, no data on electric charges on vitreous fibres have been published.

Deposition of fibres due to impaction occurs when the airflow encounters rapid changes in direction (e.g. in the nose and conducting airways) and the momentum of the fibre carries it along in a straight line to deposit on the airway wall. The larger the aerodynamic diameter, the greater the deposition efficiency due to impaction, so this mechanism is most effective for aerodynamic diameters > 0.5–1.0 μm.

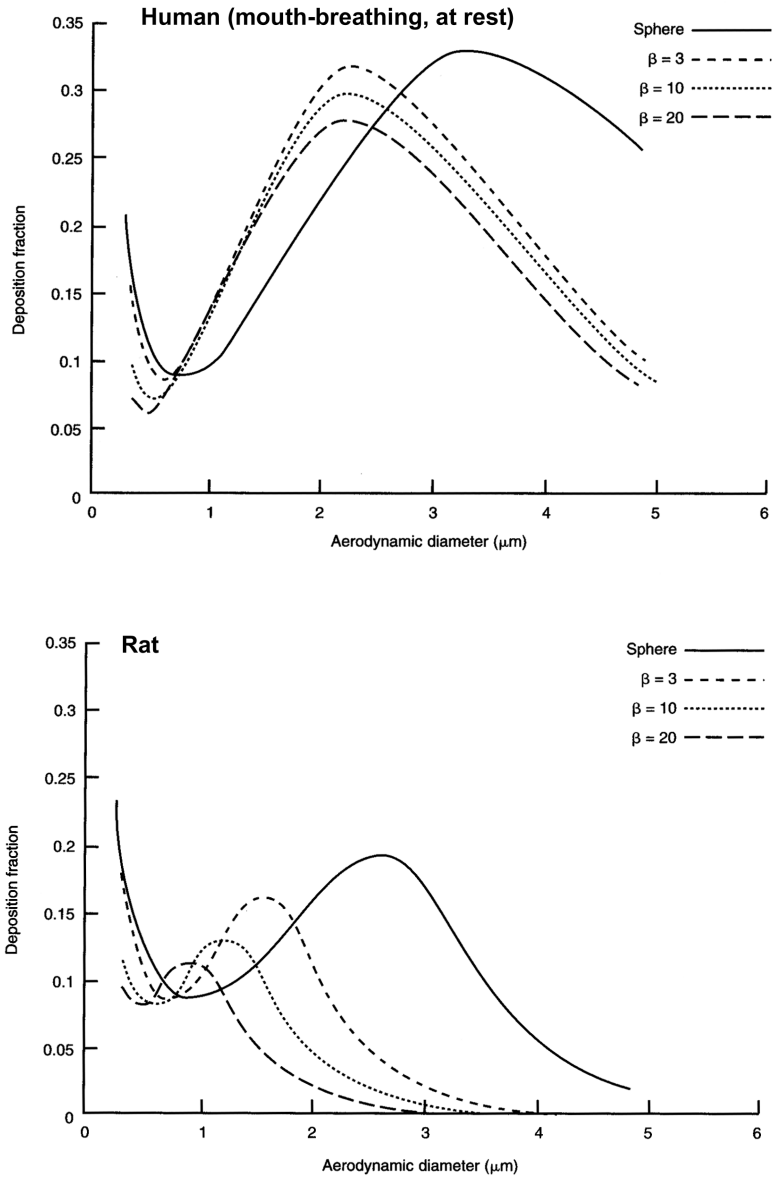
Sedimentation refers to the settling of fibres due to gravitational forces, which eventually results in the fibres touching the airway wall and depositing on the epithelium. This mechanism also operates mainly on fibres with equivalent aerodynamic diameters of > 0.5–1.0 μm.

Interception is a particularly important mechanism for fibre deposition. Whereas few spherical particles are deposited by interception, significant deposition of fibrous particles occurs by this mechanism (Asgharian & Yu, 1989). Deposition by interception occurs when an airborne particle (fibre) in the airways gets close enough to the airway wall to allow one end to touch the wall. Obviously, for an elongated object such as a fibre, this occurs more readily than for a spherical particle. In particular, fibres are carried along in the airflow while rotating at a variable rate at random orientation, even in a laminar flow (Jeffery, 1922; Asgharian & Yu, 1988), which makes interception an efficient deposition mechanism, especially for longer fibres. Only when fibres enter a laminar airflow perfectly aligned with the flow axis will they not rotate, and even then they become unaligned as soon as a bifurcation is reached.

Although these four mechanisms apply to fibre deposition in humans exposed environmentally or occupationally as well as in rodents exposed experimentally, there are important interspecies differences that need to be considered when interpreting and extrapolating results from rodent inhalation studies to humans. Figure 10 shows the differences for the alveolar region in humans and rats as modelled by Dai and Yu (1998). [The Working Group noted that there are uncertainties associated with these theoretical results.] These authors also calculated the effect of workload (increased minute ventilation) on the efficiency of deposition of fibres in humans. From the results of their model on alveolar deposition of inhaled fibres in humans, rats and hamsters, they reached the following conclusions:

- There is a significant interspecies difference in alveolar deposition of inhaled fibres, i.e. more and larger fibres deposit in humans than in rats or hamsters. This is caused by difference in the size of the structure and ventilation parameters of the airway.
- The alveolar deposition fraction in humans varies with workload. Increasing the workload reduces the deposition fraction in the alveolar region because more fibres are deposited in the ET and TB regions; switching from nose-breathing to mouth-breathing increases the deposition fraction.
- For all species, a peak in deposition occurs with particles or fibres with an aerodynamic diameter between 1 and 2 μm. Increasing the aspect ratio of the fibre

Figure 10. Predicted deposition of inhaled spherical and fibrous particles of different aspect ratios in the alveolar region of humans and rats (β)



From Dai and Yu (1998)
Specific density is 2.7 g/cm^3. Below 0.5 μm the pattern of deposition is dominated by thermodynamic properties of the particles.

results in a decrease in the peak deposition and the corresponding aerodynamic diameter.

- For rats and hamsters, alveolar deposition is essentially zero when the aerodynamic diameter of the fibres exceeds 3.5 μm and the aspect ratio is > 10. In contrast, considerable alveolar deposition occurs in humans breathing at rest, even when the aerodynamic diameter of the fibres approaches 5 μm (Dai & Yu, 1998).

The conclusion that the respirability of inhaled fibres is lower in rodents than in humans is significant in the design of experimental inhalation studies, the results of which are used for extrapolation to humans and for regulatory purposes. In particular, it would imply that it is not very meaningful to use concentrations of airborne fibres — especially long fibres — to compare the effects of exposure in humans with that in rodents. The dose in the lung is the most important consideration.

The initial deposition patterns of inhaled fibres in rats and mice have been reported by Brody and Roe (1983) and by Warheit *et al*. (1988). These authors found that the preferred site of fibre deposition in the alveolar region of rodents is on first alveolar duct bifurcations. This may also be the site of the initial inflammatory processes and perhaps for the entry of fibres into interstitial sites (see below under clearance).

The discussion in this section has focused so far on the fractions of inhaled fibres deposited in a specific region of the respiratory tract. As shown in Figure 10, deposited fractions of fibres in the alveolar region — according to the model of Dai and Yu (1998) — are significantly lower in rats than in humans so that, for a given inhaled concentration, rats apparently deposit a lower dose than humans. However, given the greater minute ventilation per unit body weight in rats, the absolute amount of deposited fibres may be higher in rats. It is, therefore, of interest to determine the amount of fibres deposited per unit surface area of the alveolar epithelium to discover whether rats or humans receive a higher dose of fibres for a given inhaled concentration. Based on these models, the fraction of fibres with an equivalent aerodynamic diameter of 2 μm and an aspect ratio of 20 deposited in the alveolar region is 2.2% in rats and 22.9% in humans (light work, mouth-breathing, Dai & Yu, 1998) (minute ventilation: 300-g rat, 245 mL/min [Hsieh *et al*., 1999]; 70-kg human, 25 L/min [ICRP, 1994]). At a concentration of 1 fibre/cm^3, 2.6×10^3 fibres are deposited in the rat and 2.75×10^6 fibres in humans over an 8-h exposure period. Assuming a lung surface area of 5.5×10^3 cm^2 in rats and 6.27×10^5 cm^2 in humans (Yu *et al*., 1995a), the dose per surface area is 0.47 fibres/cm^2 in rats and 4.38 fibres/cm^2 in humans. This is almost a 10-fold difference, and, in order for rats to deposit the same dose per surface area, the concentration to which they were exposed would have to be increased by about 10-fold.

4.1.2 *Clearance and retention*

The retention kinetics of fibres in the lung are usually influenced by the following variables: chemical composition, fibre size distribution, amount of fibres in the lung and time since exposure (Ellouk & Jaurand, 1994; Muhle *et al*., 1994; Muhle & Bellman, 1995).

Following deposition of fibres, their retention in different regions of the respiratory tract over time is determined by their clearance rate ($R = D - C$). The general mechanisms for particle clearance have been reviewed (Morrow & Yu, 1985; Oberdörster, 1988; Snipes, 1989; ICRP, 1994; Schlesinger *et al*., 1997), and models that specifically describe clearance of fibres have been published (Yu *et al*., 1996, 1998). For the nasal region, ciliary motion-mediated movement of deposited particles has been observed to occur mostly towards the pharynx and to a limited degree towards the nostrils. This is generally a fast process, but it may take up to 24 h (ICRP, 1994). Nose-blowing is a most effective means to clear the anterior region of the nose. Oropharyngeal clearance of particles or fibres deposited there occurs via the gastrointestinal tract through swallowing.

For the TB region, the main mechanism for fibre clearance is through ciliary motion along the mucociliary escalator, either after phagocytosis by airway macrophages (see below for discussion of fibre length and phagocytosis), or as free fibres. Mucociliary clearance operates throughout the TB region, where cilia are present from the trachea down to the terminal bronchioles. It is generally a fast process, which takes less than 24 h. However, the thin surfactant layer in the conducting airways of the TB region has also been found to promote the embedding of deposited spherical particles into the underlying fluid layer, essentially forcing these particles onto the epithelial cells (Schürch *et al*., 1990; Gehr *et al*., 1993). This process together with phagocytosis by airway macrophages may contribute to a long-term retention phase for spherical particles in the TB region lasting for a number of days or weeks, as observed by Stahlhofen *et al*. (1995) in humans. These authors found that the slowly-cleared fraction of the TB deposit decreased with increasing geometric diameter of the particles.

Fibrous particles may be subject to the same mechanisms, and interactions between fibres and epithelial cells would be enhanced by this process, so that translocation of persistent fibres across the TB epithelium could occur. Cigarette smoking has been found to increase the number of short asbestos fibres retained in cells of the bronchial epithelium (Churg *et al*., 1992; Churg & Stevens, 1995). Smoking-induced impairment of mucociliary clearance, an early functional abnormality in smokers, significantly reduced the clearance of deposited particles (Vastag *et al*., 1986). This could be one mechanism for the increase in risk for lung cancer in smokers exposed to asbestos (Oberdörster, 1989).

The most important mechanism for the mechanical clearance of particles deposited in the alveolar region is through phagocytosis by alveolar macrophages and subsequent

translocation towards the mucociliary escalator. Fibre length is an important parameter as it can limit the ability of alveolar macrophages to completely phagocytose a fibre. Incompletely phagocytosed fibres are likely to come into contact with alveolar cells and translocate to interstitial sites. Further interstitial transport along lymphatic channels can distribute fibres to regional lymph nodes as well as to pleural sites (Oberdörster *et al*., 1988), provided that fibres are not dissolved along this clearance pathway. The importance of mechanical and chemical processes for the overall retention of these particles in the lung is discussed in the section on biopersistence (section 4.1.3).

Clearance mechanisms in the TB region are generally the same for fibres as for spherical particles; however, there are important differences due to the elongated shape of fibres. Long vitreous fibres can break so that fibres longer than 20 μm — which are incompletely phagocytosable — become smaller and will be taken up and cleared by the alveolar macrophages.

Pleural translocation of deposited refractory ceramic fibres during and after 12 weeks of exposure by inhalation has been demonstrated in rats and hamsters. At the end of exposure and at 12 weeks after exposure, two to three times more fibres longer than 5 μm were counted in the pleural compartment of hamsters than in rats (expressed per cm^2 of pleural surface). Total pleural fibre burdens were more than three orders of magnitude lower than in the lung. This finding may partially explain the greater sensitivity of hamsters to fibre-induced pleural mesothelioma (Gelzleichter *et al*., 1999).

Important differences in the size of the macrophages between rodents and humans affect phagocytosis of fibres. In general, fibres that are too long to be phagocytosed will remain in the alveolar compartment and be subject to other clearance mechanisms, including dissolution, breakage and translocation to interstitial sites and subsequently to pleural sites. The diameters of alveolar macrophages differ between rats and humans (rat: 10.5–13 μm; human: 14–21 μm) (Crapo *et al*., 1983; Lum *et al*., 1983; Sebring & Lehnert, 1992; Stone *et al*., 1992; Krombach *et al*., 1997). Alveolar macrophages are very elastic and can spread significantly so that fibres longer than the normal diameter of an alveolar macrophage can be phagocytosed. However, phagocytosis of fibres longer than 20 μm by alveolar macrophages is less likely to occur (Dörger *et al*., 2000, 2001). Multinucleated giant cells that may have formed by fusion of macrophages upon attempted phagocytosis of longer fibres have been observed (Davis, 1970). If persistent, these longer fibres are thought to be the most potent fibre category associated with tumour induction (Kane *et al.*, 1996).

In addition to their alveolar macrophage-mediated mechanical clearance, dissolution of deposited vitreous fibres in the lung can be an important means of elimination. Short fibres that are ingested by alveolar macrophages encounter an acidic pH (4.5–5) inside the phagolysosome (Lundborg *et al*., 1995), whereas longer fibres that are incompletely phagocytosed by alveolar macrophages are exposed to extracellular fluid with a pH of ~7.4. In-vitro tests at these two pH levels (simulating the intracellular and extracellular conditions) have been developed to determine the rates of fibre dissolution (Potter &

Mattson, 1991; see section 4.1.4(a)(iii)). In-vitro dissolution and mechanical removal by alveolar macrophages are independent mechanisms: this may explain why there is not always a good correlation between in-vivo retention half-times and in-vitro dissolution rates (Oberdörster, 2000). In-vitro dissolution tests determine only the durability of fibres in a cell-free system, whereas in-vivo retention studies will determine the durability *in vivo* plus other clearance processes which together provide a measure of biopersistence of fibres (see section 4.1.3).

Poorly soluble, low-toxicity, phagocytosable particles and fibres are cleared much more slowly from the AI region of the respiratory tract than from the ET and TB regions when clearance is mediated mainly by alveolar macrophages. For rats, the normal undisturbed overall retention half-time of particles or fibres subject to macrophage-mediated clearance is 60–80 days (Snipes, 1989); for humans, the average overall retention half-time in the alveolar region is several hundred days (Bailey *et al*., 1982). In both species, the clearance rate is initially fast followed by a slow phase. In a study on human volunteers, a fast-phase retention half-time of tens of days and a slow-phase half-time of hundreds of days were reported for poorly soluble particles (Bailey *et al.*, 1982, 1985). The International Committee on Radiological Protection (ICRP, 1994) discerned three phases of particle retention in the alveolar compartment to account for the experimentally observed retention curves; however, the underlying mechanisms for each phase are not clear. It has yet to be determined whether this pattern is due to the clearance of particles deposited in the central lung regions (closer to the mucociliary escalator which starts at the level of the terminal bronchioles) being somewhat faster than that of particles deposited in the peripheral lung regions (which would have a longer clearance pathway to the terminal bronchioles) or whether it is due to the action of the different alveolar clearance mechanisms for translocation and dissolution described above. However, if a significant number of the retained particles or fibres are removed slowly, there may be serious effects on health, e.g. if the fibres are longer than 20 μm (incompletely phagocytosed, no dissolution, no breakage) or if the material is cytotoxic. In this case, prolongation of the slow phase of clearance (long retention half-time) should be considered as an indicator of potentially increased toxicity or carcinogenicity.

If the dissolution of a retained particle or fibre occurs *in vivo*, the overall retention half-time ($T_{½\,total}$) can be significantly shortened, since the clearance rates from mechanical macrophage-mediated clearance (r_{mech}) and from dissolution (r_{dissol}) are combined:

$$r_{total} = r_{mech} + r_{dissol}$$

Or, since the clearance rate and $T_{½}$ are correlated by

$$r = \frac{\ln 2}{T_{½}},$$

$T_{½\,total}$ is determined by

$$\frac{1}{T_{½\,total}} = \frac{1}{T_{½\,mech}} + \frac{1}{T_{½\,dissol}}$$

Whereas the rates of macrophage-mediated mechanical clearance are very different between rats and humans (see above), it is assumed that the rates of in-vivo dissolution of fibres are the same or very similar between species. Since $T_{½\ mech}$ is much longer in humans than in rats, a given in-vivo dissolution rate lowers the T½ total more in humans than in rodents. For example, a fibre dissolution rate corresponding to a $T_{½\ dissol}$ of 70 days will result in a $T_{½\ total}$ of 35 days for rats (half of the normal rat $T_{½\ mech}$ of 70 days), and in a $T_{½\ total}$ of 59 days (about 14% of the normal $T_{½\ mech}$) for humans (calculated using a human $T_{½\ mech}$ of 400 days) (Berry, 1999).

The dissolution of vitreous fibres occurs in extracellular and intracellular compartments, so that alveolar clearance of both long and short fibres deposited by inhalation can most often be described by a faster and a slower phase (Bernstein *et al*., 1996). Yu *et al*. (1996) developed a comprehensive model for clearance of refractory ceramic fibres from the rat lung, including macrophage-mediated translocation, dissolution and breakage, in order to describe the experimental findings on fibre accumulation from long-term inhalation studies. The inclusion of dissolution rate and breakage rate in the model provided a better interpretation of the experimental data than could be achieved with previously proposed models of fibre clearance.

Macrophage-mediated clearance can be prolonged significantly by several factors that are important for both spherical particles and fibres. These include events associated with particle overload and increased cytotoxicity of the particulate material (ILSI, 2000). In addition, the length of fibrous particles is important when it inhibits complete phagocytosis, as discussed above.

Particle overload occurs when high doses of poorly-soluble particles of low cytotoxicity are chronically deposited in the lung so that their daily rate of deposition exceeds the normal rate of macrophage-mediated clearance (ILSI, 2000). It has been shown in rats that these circumstances occurring together with retarded clearance cause persistent alveolar inflammation, fibrosis and lung tumours (Donaldson, 2000; ILSI, 2000). It has been suggested that the retarded clearance occurs when an average 6% of the volume of the alveolar macrophage is filled with phagocytosed particles, and complete cessation of clearance occurs when 60% of this volume is occupied by particles. Expressed in terms of particle mass, overload occurs when 1–3 mg of particles per gram of rat lung have been deposited (Morrow, 1988).

Other studies have suggested that the overload phenomenon correlates well with the surface area of the retained particles, which may, indeed, be a better dose parameter than particle volume or weight. It should be noted that overload-associated tumorigenic effects have been observed for poorly soluble particles of low toxicity, and only in rats. In contrast, cytotoxic materials such as crystalline silica particles show reduced alveolar macrophage-mediated clearance at lung burdens that are two orders of magnitude lower and presumably through mechanisms unrelated to overload. Impaired clearance of long fibres also involves mechanisms that do not meet the definition of lung overload (Oberdörster *et al*., 1994; Tran *et al*., 2000; Oberdörster, 2002).

The concept of lung overload is important with respect to fibres because experimental studies of inhaled fibres in rodents always include non-fibrous material and non-phagocytosable short fibres. Indeed, the non-fibrous particles, if they are biopersistent, can enhance the effects of the fibres or may contribute to chronic effects, such as fibrosis, lung tumours and mesotheliomas. For example, Davis *et al*. (1991) showed that in rats chronically exposed to asbestos fibres in combination with titania or crystalline silica particles, the incidence of lung tumours and mesothelioma was significantly increased compared with that seen with exposure to asbestos alone. Bellmann *et al*. (2001) demonstrated that RCF1 containing a large fraction of non-fibrous material induced a severe retardation of macrophage-mediated clearance of tracer particles, whereas a similar number of RCF1 fibres without the non-fibrous particles did not. The authors suggested that this was due to overload of the alveolar macrophages by the non-fibrous RCF1 particles, which could be the mechanism of lung tumour induction that had been reported in earlier long-term inhalation studies with RCF1 in rats (Mast *et al*., 1995a,b), a suggestion reiterated by Mast *et al*. (2000b). [The Working Group noted that an amount of low-toxicity non-fibrous particles equivalent to that determined to be retained in the lung by Bellmann *et al*. (2001) would not induce complete cessation of clearance of tracer particles, suggesting that particles or fibres of RCF1 have a greater cytotoxic potential than a low-toxicity particle such as titania, or that an unknown particle or fibre synergism exists. The Working Group also noted that combined exposure to particles and fibres is likely to occur at the workplace, but the ratio of particles to fibres was higher in RCF1 than that ever seen anywhere else, although not usually at levels that would induce overload (Maxim *et al*., 1997).]

The fact that lower doses of cytotoxic non-fibrous particles can cause the same chronic effects in rats as high doses of low-toxicity fibres underlines the importance of considering not only the fate of the longer, incompletely phagocytosable fibres, but also that of the shorter fibres. The latter should not induce impairment of the alveolar macrophage-mediated clearance function when the alveolar macrophage burdens are below overload. However, if the material under study were cytotoxic, similar effects to those with crystalline silica would be seen.

(*a*) *Studies in animals*

Mast *et al*. (1994) conducted inhalation studies to explore clearance of different types of refractory ceramic fibres. Rats were exposed to samples of RCF1, RCF2, RCF3 and RCF4, 1 μm in diameter and 22–26 μm in length (high purity kaolin, zirconia) and hamsters were exposed to kaolin (RCF1). For comparison, other groups of animals were exposed to chrysotile fibres. Exposure to airborne concentrations ranging between 3 and 30 mg/m^3 took place for 6 h per day on five days per week for 24 months. The lung burden of refractory ceramic fibres was found to be related to exposure: at high doses, it exceeded 10^8 fibres/g dry lung. During the various recovery periods there was a clear reduction in fibre burden. Despite the smaller airway size in hamsters compared with rats, the alveolar deposition of refractory ceramic fibres per

breathing cycle in hamsters was found to be higher than that in rats, and the calculated mean size of deposited fibres was larger in hamsters than in rats. Also, alveolar clearance of refractory ceramic fibres was faster in hamsters. The clearance rate in hamsters appeared to be independent of fibre length, but varied with the lung burden. The faster clearance rate in hamsters resulted in a lower accumulation of fibres per unit weight of the lung after a long period of exposure (Yu *et al*., 1995b).

To study the deposition and clearance of aluminium silicate ceramic fibres in the lung, Yamato *et al*. (1994a) exposed male Wistar rats to ceramic fibres with a mass median aerodynamic diameter (MMAD) of 3.7 μm for 6 h per day on five days per week for two weeks. The average concentration of fibres was 27.2 mg/m^3 (standard deviation, 9.0). The rats were examined on day 1, and at 1 month, 3 months and 6 months after the end of the exposure period, and the numbers and dimensions of the fibres were analysed by scanning electron microscopy (SEM). No significant differences in the lengths of residual refractory ceramic fibres in the lungs was found between the groups. The geometric mean diameter and number of refractory ceramic fibres, however, decreased according to the clearance period suggesting that the fibres were dissolved at their surface (see also Yamato *et al*., 1994b). Furthermore, the findings of Yu *et al*. (1994) who modelled the results of Mast *et al.* (1994) with RCF1 suggested that the clearance rate of refractory ceramic fibres from the lungs did not depend significantly upon fibre length, but that there was a clear dependence on lung burden — as lung burden increased, the clearance rate was found to decrease. Yu *et al*. (1996; 1997) used data on refractory ceramic fibres to develop a model for predicting the retention of refractory ceramic fibres in the lung of rats and humans. The model predicted that clearance of refractory ceramic fibres would not be significantly reduced in humans until the fibre concentration approaches 10 fibres/cm^3 during occupational exposure.

Rats were exposed to crocidolite or amosite asbestos or to a variety of man-made vitreous fibres (MMVFs) for five days by nose-only inhalation (6 h per day). In animals exposed to either of the asbestos types or to the more durable MMVFs (as determined by in-vitro assay; see section 4.1.4(a)), shorter fibres tended to clear from the lung more rapidly than longer fibres. However, the reverse was true for the less durable MMVFs. One year after termination of exposure, the percentages of crocidolite fibres (for which in-vivo dissolution is likely to be negligible or nil) retained in the lung were 35% for fibres < 5 μm but 83% for fibres > 20 μm in length. In contrast, the percentage retained after one year for MMVF22 slag wool (a non-biopersistent fibre) was 1% for fibres < 5 μm and < 1% for fibres > 20 μm (Hesterberg *et al*., 1996a).

In a second similar study, the percentages retained after a 30-day recovery period were 14%, 19% and 63% after treatment with amosite asbestos, RCF1a and high-alumina, low-silica (HT) (rock) stone wool fibres < 5 μm, respectively, and 60%, 50% and 3%, respectively, for amosite asbestos, RCF1a and high-alumina, low silica fibres with average lengths > 20 μm (Hesterberg *et al*., 1998b). Thus, in all cases, for the more biopersistent and slowly-dissolving fibres the long fibres disappeared more slowly than

the shorter fibres. For the less biopersistent fibres the long fibres disappeared considerably faster than the shorter fibres.

In another study in which rats were exposed to fibres by nose-only inhalation (5 days of exposure, for 6 h per day), the weighted lung-retention half-times ($WT_{1/2}$; discussed below) for eight non-biopersistent MMVFs were longer for shorter fibres than for longer fibres. For a commercial rock (stone) wool that was more biopersistent, the $WT_{1/2}$ for long fibres was the same as for short fibres (Bernstein *et al*., 1996).

The same trend was observed in a fourth study in which rats were exposed to six MMVFs by either intratracheal instillation or inhalation. The investigators observed that, for the more soluble fibres, the long fibre (> 20 μm) fractions appeared to clear faster than the shorter fibre fractions following both inhalation and intratracheal exposure (Morgan, 1994; Bernstein *et al*., 1996).

On the basis of the experimental findings, a mechanism has been proposed to explain the various length-related patterns of fibre retention. Short fibres are probably phagocytosed and transported fairly quickly by the alveolar macrophages from the lower lung up to the ciliary escalator of the upper airways and then cleared to the oral pharynx, whereas fibres > 20 μm in length are unlikely to be phagocytosed completely and cleared from the airways through similar mechanisms. The clearance of longer fibres from the lower lung would then depend upon dissolution or transverse breakage. Thus, for fibres for which dissolution is negligible (i.e. crocidolite asbestos) or very slow (traditional refractory ceramic fibres) the longer fibres disappear more slowly. The breakage of long fibres would increase the population of short fibres, while at the same time macrophage-mediated clearance would be reducing this population. Thus, the number of short fibres in the lung at any given time would be the combined result of addition by fragmentation and subtraction by translocation (Hesterberg *et al*., 1998b).

To explain the differences between clearance rates of short and long fibres, other investigators have postulated a different mechanism based on intracellular versus extracellular lung compartments. They suggested that inhaled fibres > 20 μm, which are too long for complete engulfment by macrophages, would tend to be in the extracellular compartment of the lung at near-neutral pH. The shorter fibres would tend to be in the intracellular compartment, i.e. the phagolysosomes of macrophages, where they would be subject to an acidic pH and various digestive factors (Morgan *et al.*, 1982; Christensen *et al*., 1994; Luoto *et al*., 1995, 1998). This mechanism is dealt with in more detail below and in section 4.1.4(*a*)(iv) on dissolution in in-vitro cell culture.

(*b*) *Studies in humans*

The experimental data on deposition, retention and clearance suggest that some of the parameters found to be important in rodent studies should also be taken into account in human studies.

The bivariate length:diameter distribution of fibres and the proportion of non-fibrous particles are determined by the conventional WHO (or NIOSH) phase contrast optical microscopy (PCOM) methods used in industrial hygiene (NIOSH, 1994; WHO, 1996).

Few data are available on the assessment of fibre retention in human lung tissue.

A case–control study of autopsies from the US MMVF production cohort used analytical transmission electron microscopy (TEM) to determine retention of fibres in the lung. The cases were production workers (101 in glass wool, 11 in rock (stone) and slag wool); the controls were 112 consecutive autopsies from the same hospital. The mean duration of exposure was 11 years and the mean time elapsed since the end of exposure was 12 years. No significant difference was observed in retention of MMVFs in the lung: MMVFs were detected in only 29 of the 112 production workers, of whom 14 had > 200 000 fibres/g dry lung, and 28 of the 112 controls, of whom six had > 200 000 fibres/g dry lung. Moreover, 10 of the 112 cases and two of the 112 controls had more than 1 million asbestos fibres/g dry lung ($p < 0.05$). The authors concluded that either MMVFs disappeared from the lung in less than 12 years (the numbers of fibres detected at autopsy in cases and in controls corresponded to those found after environmental exposure), or workers involved in the production of MMVFs did not inhale enough respirable MMVFs to result in a difference when compared with controls 12 years after the end of exposure, or the fixative fluids of the lung could have altered some retained fibres. They also noted that some of the MMVF production workers had also been exposed to asbestos (McDonald *et al*., 1990).

Two case reports on refractory ceramic fibre production workers, one in Europe (Sébastien *et al*., 1994) and one in the USA (INSERM, 1999), found that refractory ceramic fibres were recovered in bronchoalveolar lavage (7 cases in Europe) or in lung tissue (3 cases in the USA). All workers were still exposed to refractory ceramic fibres a few days before analysis. A significant number of refractory ceramic fibres were counted (63–764 fibres/mL in the European cases): most of the fibres had been morphologically and chemically modified (i.e. by ferruginous coating or loss of silicon and aluminium compatible with some leaching). In a review of 1800 bronchoalveolar lavage fluids from consecutive clinical series in the same chest clinic from 1992–97, pseudo-asbestos bodies on refractory ceramic fibres were detected by analytical electron microscopy in samples from nine subjects (0.5%) engaged in metal industries (foundry workers, steel workers, welders). No ferruginous bodies were detected on glass, rock (stone) or slag fibres in this series. This study also demonstrated that refractory ceramic fibres can form ferruginous bodies on durable fibres similar to asbestos. The authors concluded that refractory ceramic fibres have a residence time in the lung of at least several months, and that they interact with alveolar macrophages. Moreover, the reported presence of the typical ferruginous bodies detected by PCOM in lung, bronchoalveolar lavage fluids or induced sputum from end-users of refractory ceramic fibres should be interpreted with caution to ensure that they are not confused with asbestos bodies (Dumortier *et al*., 2001). Coated fibres have also been observed after

exposure of workers to silicon carbide fibres (Kilburn & Warshaw, 1991; Dufresne *et al.*, 1995).

4.1.3 *Fibre biopersistence, concepts and definition*

The term biopersistence as it applies to the presence of fibres in the lung was adopted to refer to the capacity of fibres to persist and to conserve their chemical and physical features over time in the lung (Hammad, 1984; Bernstein *et al.*, 1996; Hesterberg *et al*., 1998a,b; Searl *et al*., 1999; Oberdörster, 2000; Hesterberg & Hart, 2001).

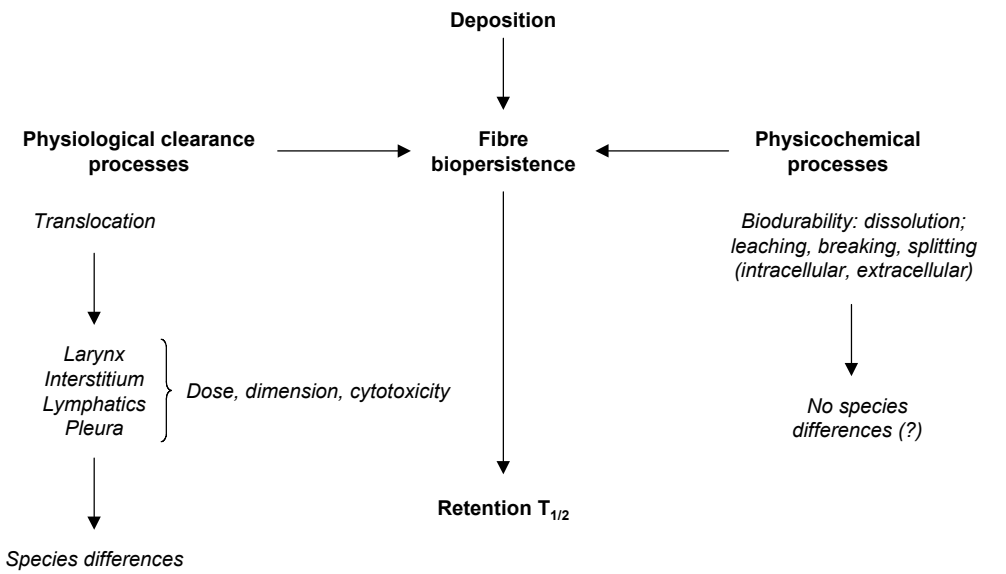
Increasing emphasis has been placed on clearance and retention of MMVFs in discussing the role of mechanistic data in risk assessment for adverse health effects induced by fibres (McClellan, 1997). This is partly because the biopersistence of fibres has been shown to play an important role in the effects on health of man-made and other mineral fibres. The retention kinetics of fibres are usually influenced by their chemical composition, size distribution and amount in the lung and may vary with time after exposure (Ellouk & Jaurand, 1994; Muhle *et al*., 1994; Muhle & Bellman, 1995, 1997). Several studies have suggested that the oncogenic potential of long MMVFs is determined by their biopersistence (Mast *et al*., 2000b; Bernstein *et al*., 2001a; Eastes & Hadley, 1996; Moolgavkar *et al*., 2001a). Pott *et al.* (1987) and Bernstein *et al*. (2001a,b) suggested that a certain minimum persistence of long fibres is necessary before early changes start to appear in the lung. Furthermore, Moolgavkar *et al*. (2001b) have suggested, on the basis of their model, that fibre-induced cancer risk, in addition to being a linear function of exposure concentration, is also a linear function of weighted half-time of the fibres. There are also significant interspecies differences in cancer susceptibility. Although comparisons between species may be difficult, rats are considered to be the preferable model for assays of toxicity and oncogenicity for particulate materials (ILSI, 2000). In view of the increasing body of experimental evidence for the role of biopersistence in fibre toxicity, it is not surprising that several attempts have been made to reduce the biopersistence of fibres by increasing their biosolubility through changes in the chemical composition of the raw material of the fibres (Guldberg *et al*., 2000).

The influence of fibre length on fibre toxicity was first shown for asbestos fibres. Vorwald *et al*. (1951) showed that long fibres (20–50 μm) were associated with both lung and peritoneal disease whereas shorter, ball-milled fibres (3 μm or less) were not. The importance of the in-vivo durability of a fibre and of fibre dimension in the pleural and intraperitoneal cavities was determined in the early studies of Stanton & Wrench (1972) and Pott *et al*. (1974). Long, thin and durable fibres have the greatest potential to cause tumours. In an early study on the biopersistence of synthetic mineral fibres, Hammad (1984) found that retention of refractory ceramic fibres < 5 μm in length was relatively low following short-term inhalation. With longer fibres, retention increased sharply, reached a peak at a fibre length of 11 μm, and then

decreased again; fibres with lengths > 30 μm cleared most slowly. A less durable fibre tested in the same study cleared more rapidly. This knowledge provides the basis for the concept of fibre biopersistence: that is, the longer a fibre persists in the lower respiratory tract, the greater its likelihood to cause effects, especially if it is longer than 20 μm (Bernstein *et al*., 1996; Hesterberg *et al*., 1998a,b).

The scheme in Figure 11 summarizes the different processes that contribute to the biopersistence of a fibre (Oberdörster, 2000). The pulmonary retention half-time of a fibre, determined in an in-vivo study, reflects its biopersistence, which is determined by elimination due to physiological clearance processes such as translocation to the larynx by alveolar macrophages, into the interstitium, via the lymphatic system and the pleura, and to physicochemical processes that affect biodurability, such as dissolution, leaching and breaking. As discussed in section 4.1.2, physiological clearance rates such as those for macrophage-mediated removal are very different between humans and rodents; in contrast, it is assumed that physicochemical processes occur at similar rates. The breakage of incompletely phagocytosed long fibres into shorter fragments can decrease their biopersistence significantly; at the same time, the shorter fragments enter the pool of the short fibres, which can result in an apparent increase in their biopersistence.

Figure 11. Factors contributing to the biopersistence of fibres in the lung



Modified from Oberdörster (1996)

The goal of a biopersistence assay is to assess the potential for accumulation of fibres after long-term exposure. However, it should be noted that the retention time for a fibre derived from a short-term (e.g. 5-day) inhalation assay may be lower than the value determined from a subchronic or chronic inhalation study. Therefore, a retention time determined from a short-term assay should not be used to predict accumulation of fibres in the lung during a long-term study. Various methods are available to determine the retention of fibres in the respiratory tract. Short-term (5-day) inhalation studies in rats have been designed for the determination of fibre retention (Bernstein *et al*., 1994; Bernstein & Riego-Sintes, 1999). In order to exclude the influence of mucociliary clearance, measurements of retention are generally started some time after exposure. A two-phase pattern of retention is generally observed for the three categories of fibre length, i.e. < 5 μm, 5–20 μm and > 20 μm. For the shorter, phagocytosable fibres, the underlying mechanisms for these phases should be similar to those described for alveolar clearance of non-fibrous particles (see section 4.1.2), i.e. involving clearance pathways of different lengths from the site of deposition by translocation to the mucociliary escalator as well as dissolution in alveolar macrophages; for long fibres, dissolution and breakage can be assumed to be the main mechanisms to explain the two-phase clearance.

(*a*) *Fibre biopersistence studies* (see Table 65)

In the absence of highly toxic elements or surface structures, a more biopersistent fibre will have a greater potential to induce biological effects in the lung. In addition, biopersistent fibres are more likely than rapidly-clearing fibres to be taken up by the lung epithelium and translocated into the interstitium and on to the pleura and thoracic cavity, where they would continue to cause irritation and inflammation. Thus, a more biopersistent fibre may have an impact on a broader range of target tissues. For example, the overall retention of glass fibres *in vivo* depends not only on their chemical composition, but also on their length. This effect has been attributed to differences in the microenvironment to which long and short fibres are exposed. Although this phenomenon appears to operate with all the glass fibres examined, it does not apply to the MMVFs that dissolve more readily in environments with low pH (Morgan, 1994; Baier *et al*., 2000).

Overall, recent chronic studies of vitreous and asbestos fibres conducted in rodents indicate a relationship between persistence of fibres in the lung and the severity of their biological effects (for reviews, see Bernstein *et al.*, 2001a,b; Hesterberg & Hart, 2001).

After deposition in the lung, biopersistent fibres such as crocidolite or amosite asbestos exhibited little or no change in chemical composition, surface morphology or dimension (suggesting no significant leaching, dissolution or transverse fragmentation), whereas fibres with lower biopersistence such as MMVF10 and MMVF11 and the newly developed high-alumina, low-silica (HT) stone wool did show changes as follows:

— compositions changed and surfaces often showed signs of erosion;

Table 65. Lung biopersistence, in-vitro dissolution and pathogenicity of selected fibres from inhalation studies in rats

Fibre		Biopersistence: fibres > 20 μm in length; lung clearance rates				In-vitro dissolution (k_{dis}) at pH 7.4 (pH 4.5)*	Pathogenicity (chronic inhalation)		
		Slower pool (T½)	$WT_{½}$ (days)	90% clearance (T_{90}, days)	Reference		Lung fibrosis	Thoracic tumours	Reference
Amosite	Asbestos	1160	418	2095	Hesterberg *et al.* (1998a)	< 1	+	+	McConnell *et al.* (1999)
Crocidolite[d]	Asbestos	0	817	2770	Hesterberg *et al.* (1996a)	< 1	+	+	McConnell *et al.* (1994)
MMVF32	E Glass wool	179	79	371	Hesterberg *et al.* (1998a)	9 (7)	+	+	Davis *et al.* (1996a)
RCF1a[a]	Refractory ceramic fibre	88	55	227	Hesterberg *et al.* (1998a)	3	+	+	Mast *et al.* (1995a,b)
MMVF33	475 Glass wool	155	49	240	Hesterberg *et al.* (1998a)	12 (13)	+	+/–[b]	Davis *et al.* (1996a); McConnell *et al.* (1999)
MMVF21	Rock (stone) wool (96)	613	91	206	Hesterberg *et al.* (1996a)	20 (72)	+	–	McConnell *et al.* (1994)
MMVF21	Rock (stone) wool (98)	95	67	264	Hesterberg *et al.* (1998a)				
MMVF10[d]	901 Glass wool (96)	0	37	123	Hesterberg *et al.* (1996a)	300 (329)	–	–	Hesterberg *et al.* (1993)
MMVF10.1[c]	901 Glass wool	30	14.5	69	Hesterberg *et al.* (2002)		–	–	McConnell *et al.* (1999)

Table 65 (contd)

Fibre		Biopersistence: fibres > 20 μm in length; lung clearance rates				In-vitro dissolution (k_{dis}) at pH 7.4 (pH 4.5)*	Pathogenicity (chronic inhalation)		
		Slower pool (T½)	$WT_{½}$ (days)	90% clearance (T_{90}, days)	Reference		Lung fibrosis	Thoracic tumours	Reference
X607	Hybrid fibre	94	10	18	Bernstein *et al.* (1996)	990	–	–	Hesterberg *et al.* (1998a)
MMVF11	Glass wool	31	9	38	Hesterberg *et al.* (1996a)	100 (25)	–	–	Hesterberg *et al.* (1993)
MMVF22	Slag wool	35	9	37	Hesterberg *et al.* (1996a)	400 (459)	–	–	McConnell *et al.* (1994)
MMVF10B	901F Glass wool	20	8	38	Hesterberg *et al.* (2002)	500	ND	ND	
MMVF35	902 Glass wool	18	7	33	Hesterberg *et al.* (2002)	150	ND	ND	
MMVF34	HT Stone wool	24	6	19	Hesterberg *et al.* (1998a)	59 (620)	–	–	Kamstrup *et al.* (1998)

From Hesterberg *et al.* (2002); biopersistence data based on 5-day inhalation studies; pathogenicity based on chronic rodent inhalation studies
ND, no data (chronic toxicity not evaluated); $T_{½}$, retention half-time of slow clearance phase (if biexponential) or of one clearance phase (if monoexponential); $WT_{½}$, weighted retention half-time; T_{90}, number of days to clear 90% of fibres, calculated from model using estimated fibres/lung directly after exposure (rather than on day 1 of recovery); k_{dis}, ng/cm^2/h; minus sign (–) indicates no fibrosis or tumour incidence not significantly different from background incidence in the test species.
* From Zoitos *et al.* (1997); Hesterberg *et al.* (1998a,b); reviewed in Hesterberg & Hart (2001)
[a] RCF1a (biopersistence studies) was modified from RCF1 (chronic studies) to contain fewer non-fibrous particles.
[b] +/– indicates tumorigenicity in hamsters (one mesothelioma in 83 animals), but not in rats; Hesterberg *et al.*, 1998a; Davis *et al.*, 1996a.
[c] MMVF10.1 was size-selected from MMVF10 to have longer and thinner average dimensions. The original MMVF10 was used for previously published chronic and biopersistence studies (Hesterberg *et al.*, 1993; 1998b).
[d] Monoexponential retention curve

— fibre dimensions decreased; and
— the number of long fibres per lung decreased more rapidly than the number of short fibres per lung (suggesting transverse fragmentation)

(Bernstein *et al*., 1984; Morgan & Holmes, 1986; Musselmann *et al*., 1994a,b; Hesterberg *et al*., 1996b, 1998b).

In two studies, rats were exposed by nose-only inhalation to high concentrations of one of eight different MMVFs or to one of two types of asbestos for five days (6 h per day) and were then kept without further exposure. After post-exposure intervals of up to one year, five to seven animals per exposure group were killed and their lung fibre burdens were evaluated. (Hesterberg *et al*., 1996c, 1998b).

In the first of these studies, rats were exposed to two standard glass wools used in building insulation (MMVF10 and MMVF11), slag wool (MMVF22) or traditional rock (stone) wool (MMVF21) at approximately 250–350 WHO fibres/cm^3 > 5 μm (including ~100 fibres > 20 μm/cm^3) or to crocidolite asbestos at approximately 2600 WHO fibres/cm^3 > 5 μm (including ~290 fibres > 20 μm/cm^3) (Hesterberg *et al.*, 1996c). In the second study, rats were exposed to a refractory ceramic fibre (RCF1a), a traditional rock (stone) wool (MMVF21), JM E glass wool (a special application microfibre) (MMVF32), JM 475 glass wool (a special application microfibre) (MMVF33) or the newly developed high-alumina, low-silica (HT) stone wool (MMVF34) at ~400 WHO fibres/cm^3 (including ~150 fibres > 20 μm/cm^3) or to amosite asbestos at ~800 WHO fibres/cm^3 > 5 μm (including 235 fibres > 20 μm/cm^3) (Hesterberg *et al.*, 1998b). The MMVFs tested had approximate arithmetic mean dimensions of 1 μm × 20 μm and geometric mean dimensions (GMD) of 0.7–0.9 μm diameter and 12–16 μm length. The crocidolite fibres were smaller than the MMVFs (GMD, 0.3 μm × 4.2 μm), but the amosite fibres were more comparable in size (GMD, 0.5 μm × 7.7 μm).

The rat lungs were analysed at intervals during a one-year post-exposure recovery period for numbers of fibres in the lung, dimensions, morphology and chemical composition. In both of these biopersistence studies, the deposition in the lung of long fibres (> 20 μm) was roughly comparable for the eight MMVFs and the two types of asbestos (crocidolite and amosite) as measured by the lung burden one day after cessation of exposure. However, the deposition of shorter fibres was greater for asbestos than for the MMVFs. The lung burdens of the MMVFs were more similar to those of amosite than to those of crocidolite (both in numbers and dimensions).

In both studies, asbestos fibres cleared much more slowly than the MMVFs: $WT_{1/2}$ retention times for fibres > 20 μm were 817 days for crocidolite and 418 days for amosite, compared with 6–67 days for MMVFs. After one year of recovery, retention of fibres > 20 μm in the lung was 83% for crocidolite and 30% for amosite, compared with 0–10% for the MMVFs.

The different MMVFs had a broad range of retention times: $WT_{1/2}$ values were 49–79 days for the slower-clearing MMVFs (rock (stone) wool, the two special-application glass wools and refractory ceramic fibre), and only 6–9 days for the more rapidly-clearing MMVFs (the two standard building-insulation glass wools, slag wool and high-

alumina, low-silica (HT) stone wool). Of all fibre compositions and length categories tested in both of these biopersistence studies, the number of HT rock (stone) wool fibres > 20 μm per lung underwent the most rapid reduction. By 30 days post-exposure, the lung burdens of HT fibres > 20 μm were 3% of those measured on day 1 and by 180 days, they had been reduced to background levels (Hesterberg *et al*., 1998b).

For asbestos and the more biopersistent MMVFs, the number of short fibres tended to decrease more rapidly than the number of long fibres. The investigators suggested that this was a likely result of macrophage-mediated lung clearance, which is more efficient for shorter fibres. However, for the more biosoluble MMVFs, the numbers of long fibres decreased more rapidly than the number of short fibres. The investigators suggested that this could be a result of a rapid rate of transverse breakage of long, biosoluble fibres into short fibres. Transverse fragmentation of fibres has been demonstrated *in vitro* for rapidly leaching compositions of MMVF (Bauer, 1998a).

During the year of post-exposure recovery, changes in the dimensions of the fibres in the lung were noted. For the less biopersistent MMVFs, mean lengths decreased, suggesting transverse breakage and/or dissolution. The mean lengths of fibres of crocidolite and amosite increased, suggesting selective clearance of shorter fibres by macrophages; their diameters remained unchanged.

Analysis of HT rock (stone) wool fibres deposited in the lung by energy dispersive spectroscopy showed a decrease in the percentage of calcium oxide after the first 30 days of post-exposure recovery (Hesterberg *et al*., 1998b). After 91 and 365 days of residence in the lung, the percentages of alkali oxides (Na_2O) and alkaline earth oxides (magnesium oxide and/or calcium oxide) in MMVF10, MMVF11 and MMVF22 showed significant decreases, while MMVF21 and crocidolite showed no change in chemical composition (Hesterberg *et al*., 1996c).

Scanning electron microscopy (SEM; using magnification up to × 50 000) was used to examine the morphology of fibres in the lung at several time points up to three months post-exposure. At one extreme was amosite, in which no morphological changes were observed in any of the fibres in the lung during the three months of monitoring. Most of the refractory ceramic, rock (stone) wool and JM E glass fibres (MMVF32) also underwent no visible changes during the three months. The JM 475 glass fibres (MMVF33) showed a range of morphological changes in the lung, from negligible to fairly severe surface etching. At the other extreme were the slag wool and HT stone wool fibres, most of which developed severe surface etching and other visible indications of deterioration starting as early as one week post-exposure. Common findings in HT stone wool fibres were crater-like surface pits, rounded or blunt ends, dissolved cores and segmented fibre fragments lying end-to-end on the SEM stub filter, the latter indicating transverse breakage during the final processing of samples.

Changes in the numbers of fibres and in their chemistry, dimensions and/or morphology during residence in the lung were more pronounced for the eight MMVFs than for the two types of asbestos. These changes were especially striking in the four MMVFs that cleared most rapidly in the studies of biopersistence, did not produce lung

fibrosis or tumours and were non-pathogenic in the chronic studies (the two insulation glass wools, slag wool and HT stone wool). The induction of fibrosis and tumours by a specific fibre type was found to be closely associated with its biopersistence and to a lesser extent with its in-vitro rate of dissolution at pH 7.4 (discussed below). The investigators concluded that these observations supported the hypothesis that the biopersistence of inorganic fibres in the lung is a major determinant of fibre toxicity and is determined in part by the rate of fibre dissolution (Hesterberg *et al*., 1998b).

The biopersistence of nine MMVFs (four glass wools, four rock (stone) wools and X-607) was evaluated in rats exposed to the fibres for five days (6 h per day) by nose-only inhalation. The fibres included in the study were both commercial and experimental compositions. After various post-exposure time periods, the whole lung was removed and ashed and the fibres from the lung were analysed. At the end of the period of exposure, the lung burdens for the nine fibres ranged from 5.7–33 × 10^6 fibres/lung and fibres had geometric mean diameters of ~0.5 μm. The $WT_{1/2}$ of the nine fibres was 11–54 days for WHO fibres and 2.4–45 days for fibres > 20 μm. For one of the fibres, a commercial rock (stone) wool, the number of longer fibres (> 20 μm) per lung declined more rapidly than that of shorter fibres. The $WT_{1/2}$ of the nine fibres correlated quite well with their rates of in-vitro dissolution (Bernstein *et al*., 1996).

Groups of male Fischer 344 rats were exposed to six MMVFs (three glass wools, two rock (stone) wools and RCF1a) by either nose-only inhalation (5 days, 5 h per day; target aerosol concentration, 30 mg/m^3) or by intratracheal instillation (0.5 mg fibres in 0.3 mL saline per day for 4 days). Subgroups of rats were killed after various post-exposure intervals, the whole lung was ashed and fibres recovered from the lung were analysed. For all the glass wools and rock (stone) wools, longer fibres (> 20 μm) cleared more rapidly than WHO fibres (> 5 μm); for RCF1a, the rate of clearance of both size fractions was about the same. Following intratracheal instillation, the $WT_{1/2}$ of fibres > 20 μm for the glass and rock (stone) wools varied between 3 and 12 days, but RCF1a had a $WT_{1/2}$ of > 1000 days. Following inhalation exposure, the $WT_{1/2}$ of fibres > 20 μm was 4–6 days for the glass and rock (stone) wools, but 64 days for RCF1a. The authors suggested that the very long $WT_{1/2}$ seen for RCF1a seen after intratracheal instillation may have been due to agglomeration in the bronchi (Bernstein *et al*., 1997).

Rats received eight different types of mineral fibre by intratracheal injection, and the relative biopersistence of the fibres in different size categories was assessed from the changes in mean lung burden, as determined by SEM, at three days and one, six and 12 months after instillation. The samples tested included the commercially available glass fibre 100/475, six size-selected fibre types specifically prepared for research purposes (MMVF10, MMVF21, MMVF22, RCF1, RCF2 and RCF4), and amosite. Short fibres were cleared by cellular processes and long fibres by dissolution and disintegration. The differences in persistence of long fibres (> 20 μm) of these fibre types were correlated with the rates of dissolution measured *in vitro*. The differences in persistence noted for those fibre types that had also been studied by other research groups were consistent with their findings, both after administration of fibres by

inhalation and intratracheal injection. The biopersistence of the different fibres was influenced by their dimensions and solubility. For the six more durable compositions tested (amosite, 100/475, MMVF21, RCF1, RCF2 and RCF4), long fibres tended to clear from the lung more slowly than short fibres. For the two less durable fibres (MMVF10 and MMVF22), in contrast, longer fibres cleared more rapidly than shorter ones (Searl *et al*., 1999).

(*b*) *Fibre biopersistence and pathogenicity*

Several short-term cellular tests for predicting the pathological effects of particulates and fibres are reported in sections 4.2–4.5, but the results often do not correlate with the results of studies on biopersistence *in vivo*. This indicates that, as documented in a recent workshop report, several chemical and biological properties in addition to biopersistence have to be considered in order to screen for pathogenicity of fibres (Fubini *et al*., 1998).

In an early study, male Fischer 344/N rats were exposed to size-separated respirable fractions of glass fibre with compositions representative of common building-insulation wools. The animals were exposed in nose-only inhalation chambers for 6 h per day on five days per week for 24 months to either 3, 16 or 30 mg/m^3 of two different compositions of glass fibre (MMVF10 and MMVF11). Filtered air was used as a negative control treatment. The findings for glass fibre were compared with those from a concurrent inhalation study of chrysotile asbestos and refractory ceramic fibre. Fibres were recovered from digested lung tissue for the determination of changes in fibre number and morphology. In animals exposed to 30 mg/m^3 MMVF10 or MMVF11, 4.2×10^8 and 6.4×10^8 fibres/g dry lung tissue, respectively, were recovered after 24 months of exposure. For rats exposed to refractory ceramic fibres and chrysotile these numbers were 3.7×10^8 and 1.9×10^{10} fibres/g dry lung, respectively. Exposure to chrysotile asbestos (10 mg/m^3) and to a lesser extent to refractory ceramic fibres (30 mg/m^3) resulted in pulmonary fibrosis as well as mesothelioma and significant increases in the incidence of lung tumours. Exposure to glass fibre was associated with an inflammatory macrophage response in the lungs that did not appear to progress after 6–12 months of exposure. These cellular changes were reversible. No lung fibrosis was observed in the animals exposed to glass fibre, and neither mesotheliomas nor statistically significant increases in the incidence of lung tumours were observed, when compared with the negative-control group (Hesterberg *et al.*, 1993).

Inhalation studies (McConnell, 1994; McConnell *et al.*, 1994; Mast *et al*., 1995a,b; McConnell *et al.*, 1995) were also conducted in rodents to determine the chronic biological effects of rodent-respirable fractions of MMVFs including refractory ceramic fibres, glass fibre, rock (stone) wool (MMVF21) and slag wool (MMVF22). Animals were exposed by nose-only inhalation for 6 h per day on five days per week for 18 months (Syrian golden hamsters) or 24 months (Fischer 344/N rats). Exposure to 10 mg/m^3 of crocidolite or chrysotile asbestos induced pulmonary fibrosis, lung tumours and mesotheliomas in rats and hamsters, in these chronic inhalation studies.

Exposure of rats to 30 mg/m^3 refractory ceramic fibres also resulted in pulmonary fibrosis and pleural fibrosis as well as a significant incidence of lung tumours and mesotheliomas. In hamsters, 30 mg/m^3 refractory ceramic fibres induced a 41% incidence of mesotheliomas. Exposure of rats to 30 mg/m^3 glass fibre (MMVF10 or MMVF11) or slag wool (MMVF22) was associated with an inflammatory response, but no mesotheliomas or lung tumours were observed after 12 or 24 months of exposure. The same dose of rock (stone) wool (MMVF21) resulted in minimal lung fibrosis and no malignant tumours.

In a chronic inhalation study, male Wistar rats were exposed to an E-glass microfibre (104E; a special-purpose fibre) at a concentration of 1000 fibres > 5 μm/mL of air for 7 h per day on five days per week for 12 months. After a 12-month post-treatment recovery period, the retained lung burden (of fibres of all lengths) was about 30% compared to the burden immediately after treatment. Amosite asbestos and 100/475 microfibres, which were used as controls, were retained at levels of 44% and 28%, respectively. The 104E fibres were thus slightly less persistent in the lungs than amosite. The chemical composition of the 104E fibres did not change during their 24-month residence time in the lungs, but that of the 100/475 microfibres did. Exposure of rats to 104E microfibres and amosite asbestos by inhalation markedly increased the incidence of lung tumours (carcinomas and adenomas) and mesotheliomas and induced pulmonary fibrosis. In contrast, in the animals treated with 100/475, little fibrosis, a few lung adenomas, no carcinomas, and no mesotheliomas were observed (Cullen *et al*., 2000). [The Working Group noted that the low number of fibres counted may explain some anomalies in the lung fibre burdens.]

Fischer 344 rats were exposed by nose-only inhalation to MMVF21 (traditional rock (stone) wool) and MMVF34 (HT stone wool), for 6 h per day for five days, and then followed post-exposure for up to 12 months. In a second study, rats were exposed for 6 h per day for two years with a post-exposure follow-up until survival in the exposed group was approximately 20%. The short-term study used concentrations of 150 fibres (> 20 μm)/cm^3 and the long-term study 30 mg/m^3. In both these studies, the biopersistence pattern for MMVF34 was similar. The biopersistence of MMVF34 was less than that of MMVF21 when elimination half-lives after short-term inhalation were compared. [The Working Group noted that the weighted half-times ($WT_{1/2}$) reported in the studies by Hesterberg *et al*. (1998b) and Kamstrup *et al*. (1998) increased from six to 17 and 27 days following inhalation for five days, three months and 12 months, respectively.] Only minor histopathological changes were observed in the lungs of rats exposed to MMVF34 compared with those exposed to MMVF21 after up to 18 months. At this time, MMVF21, but not MMVF34, had induced lung fibrosis. From the preliminary histopathological results the authors considered it unlikely that animals exposed to MMVF34 would develop pulmonary tumours in excess of control incidences (Kamstrup *et al*., 1998).

4.1.4 *Fibre dissolution*

(*a*) *In-vitro dissolution* (see Table 65)

(i) *Cell-free systems*

The physical and chemical mechanisms whereby fibres may degrade in the lung have been studied extensively *in vitro* using balanced salt solutions ('simulated lung fluids') (Law *et al*., 1990, 1991; Christensen *et al*., 1994; Luoto *et al*., 1994a,b; de Meringo *et al*., 1994; Hesterberg *et al*., 1996a; Knudsen *et al.*, 1996; Bauer, 1998a,b). Because fibre dissolution and breakdown in the lung are important determinants of lung clearance, in-vitro dissolution tests have been suggested for screening for toxicity of fibres (Scholze & Conradt, 1987; Eastes & Hadley, 1996).

[The Working Group noted that dissolution of solids may be strongly influenced by the presence of surfactant and molecules that selectively bind to surface ions. Ascorbic acid, which is one of the major antioxidant defences in the lung lining layer, makes crystalline silica more soluble than amorphous silica (Fenoglio *et al.*, 2000).]

The dissolution of silica from a range of industrial MMVFs (including glass wool, rock (stone) wool, slag wool and refractory ceramic fibres) was compared *in vitro* with that of crocidolite, using a solution similar to that of Gamble (1967). The calculated reductions in diameters (based on chemical analysis of the effluent) for the MMVFs ranged from 0.2–3.5 nm/day. The corresponding value for crocidolite was < 0.01 nm/day. The rates of dissolution for glass fibres showed a 15-fold variation; the solubility of samples of rock (stone) wool and slag wool (composition specified) was intermediate among the fibres tested; and the solubility of the refractory ceramic fibres was generally at the lower end of the range (Scholze & Conradt, 1987). Leineweber (1984) also found great variability in the solubility of glass fibres (a 30-fold range in the dissolution rate constants (k_{dis}), from 3.0 to 0.1 ng/cm^2/h); a refractory ceramic fibre was found to be fairly insoluble (0.4 ng/cm^2/h). These k_{dis} values are much lower (by about one order of magnitude) than those measured by Zoitos *et al*. (1997) and other recent investigators. The dissolution rates (in ng/cm^2/h ± SD) were reported for the following fibres: MMVF21 (23 ± 11), MMVF22 (119 ± 41), MMVF11 (142 ± 39), MMVF10 (259 ± 75) and RCF1 (8 ng/cm^2/h; no SD given). Crocidolite has a very low dissolution rate (0.3 ± 0.1). It is assumed that the experimental methods or the sensitivity of the analytical method — comparing weights of residual fibre samples to weights of initial fibre samples — may account for these discrepancies.

Recent studies of in-vitro dissolution and degradation have typically employed a flow-through dissolution system based on the work of Klingholz & Steinkopf (1984) and Leineweber (1984), in which physiological fluid (similar to that described by Gamble, 1967) is passed through a fibre sample. At various time-points, the effluent from the flow-through system is analysed for dissolved fibre components. A dissolution constant (k_{dis}, ng/cm^2/h) is then estimated from the effluent data (Potter & Mattson, 1991). The flow rate must be rapid enough to clear the dissolution products from the fibre surface, but not so rapid as to dilute the dissolution products so much

that they cannot be analysed accurately. It is also important to note that silica and aluminium compounds vary widely in the extent to which they are involved in the leaching process. This makes analysis of a single component in the effluent unsuitable as a means of comparing the dissolution rates of a wide range of compositions (Mattson, 1994).

A broad range of in-vitro dissolution rate constants (k_{dis}, ng/cm^2/h) were observed for a variety of inorganic fibres tested in a flow-through system at near-neutral pH (i.e. pH 7.4–7.6 which simulates the pH of extracellular fluid). The rates were as follows: < 1 for crocidolite asbestos; 8–12 for biodurable MMVFs, such as refractory ceramic fibres, E-glass microfibre and 475 glass microfibre, and 100–300 for soluble MMVFs, such as building-insulation glass wools and slag wool (Zoitos *et al*., 1997; Hesterberg *et al*., 1996a, 1998a). Some fibres have much higher k_{dis} values; for example, JM 909 building-insulation glass fibre which has a k_{dis} > 1000 (Hesterberg & Hart, 2001) and various new or experimental compositions of rock (stone) wool and glass wool which have a k_{dis} > 500 (Hesterberg *et al.*, 1998b). As noted by Hesterberg *et al*. (1998b), with rare exceptions, the values for k_{dis} for MMVFs at pH 7.4 have shown a good correlation with in-vivo lung clearance and with pathogenicity.

Dissolution rates have also been studied using fluid at pH 4.5, which simulates the acidic pH of the phagolysosome of macrophages (Etherington *et al*., 1981). Sébastien (1994) found a good correlation between fibre clearance (from the rat lung following intratracheal instillation) and the in-vitro dissolution rate, but only if k_{dis} values at both pH 7.7 and pH 4.5 were taken into consideration. Other researchers have stressed the importance of using dissolution rates at both pHs to predict the in-vivo biopersistence of a fibre (Guldberg *et al*., 1998, 2000). The HT stone wool (MMVF34) is a case in point: it was observed to be non-persistent in the rat lung (weighted retention half-time of fibres > 20 μm, 6 days; see above), but it did not dissolve rapidly *in vitro* at near-neutral pH (k_{dis} at pH 7.4, 59; Hesterberg *et al*., 1998b). However, HT did dissolve rapidly at acidic pH (k_{dis} at pH 4.5, 1100) (Knudsen *et al.*, 1996; Guldberg *et al*., 1998).

The in-vitro dissolution of seven traditional MMVF compositions was tested. The fibres studied were JM 475/100 glass wool microfibre, building-insulation glass wool (MMVF10), rock (stone) wool (MMVF21), slag wool (MMVF22), three refractory ceramic fibres (RCF1, RCF2 and RCF4)) and amosite asbestos. Fibres were subjected to either a static system (sodium oxalate at initial pH 4.6 or pH 7.0 for 56 days) or a flow-through system (Kanapilly's solution, similar to Gamble's, at pH 7.4). Higher in-vitro dissolution rates of these fibres corresponded with lower biopersistence in rat lung following either intratracheal injection or inhalation of fibres (see above); MMVF10 and MMVF22 were the least durable. After 56 days of treatment with sodium oxalate, the percentage of silica dissolved at pH 4.6 was 31% for MMVF10, 51% for MMVF22, but < 1–6% for all other fibres; dissolution of silica at pH 7.0 was 15% for MMVF10 and 53% for MMVF22, but < 1–6% for all others. Flow-through k_{dis} values (ng/cm^2/h) at pH 7.4 were: 122 and 53 for MMVF10 and MMVF22,

respectively, but 0.5–29 for the other fibre types (k_{dis} for amosite not reported) (Searl *et al*., 1999).

The effect of different chemical compositions of MMVF on their dissolution by alveolar macrophages in culture and in cell-free Gamble's solution was studied. The fibres were exposed to cultured rat alveolar macrophages, culture medium alone, or in Gamble's saline solution for 2, 4 or 8 days. The dissolution of the fibres was studied by measuring the amount of silicon (Si), iron (Fe), and aluminium (Al) in each medium. The macrophages in culture dissolved Fe and Al from the fibres, but the dissolution of Si was more marked in the culture medium without cells and in the Gamble's solution. The dissolution of Si, Fe, and Al was different for different fibres, and increased as a function of time. The Fe and Al content of the fibres correlated negatively with the dissolution of Si from the MMVFs by macrophages, i.e. the higher the content of Fe and Al of the fibres the lower the dissolution rate of Si. The results suggest that the chemical composition of the MMVF has a marked effect on its dissolution. Alveolar macrophages seem to affect the dissolution of Fe and Al from the fibres. This suggests that in-vitro models with cells in the media rather than cell-free culture media or saline solutions would be preferable in dissolution studies of MMVFs (Luoto *et al.*, 1994a).

Glass fibres with a mean diameter of 1 μm of different chemical composition and obtained by different processes were characterized with respect to their solubility under various test conditions and flow rates. The surface morphology was examined using SEM and energy dispersive spectroscopy. The SEM revealed the typical formation of various corrosion patterns: porous, gel-like outer layers, precipation zones, but, in some cases, no modification of the surface aspect was seen. The results show that surface changes depend strongly on the initial composition of the glass and on the test conditions, particularly the flow rate (Lehuédé & de Meringo, 1994).

To evaluate in-vitro dissolution of fibres, JM 475/100 microfibre glass wool and amosite asbestos were suspended and incubated in sodium oxalate (pH 4.6 or 7.0) or in 0.1 M oxalic acid (pH 0.6), and the silicon content of the supernatants was determined at intervals up to 56 days. Changes in pH were found to be minimal over this period. After 56 days of incubation in sodium oxalate at either pH 4.6 or 7.0, JM 475/100 lost about 6% of its silicon content, while amosite lost only 1–2%. After 28 days of incubation in oxalic acid, JM 475/100 lost 29% of its silicon, while amosite lost only 9% (Davis *et al*., 1996a).

Several researchers have stressed the importance of establishing standardized methods for estimating in-vitro dissolution rates (Mattson, 1994; Zoitos *et al*., 1997; Guldberg *et al*., 1998). There is general consensus that a flow-through system similar to that described by Potter and Mattson (1991), with a modified Gamble's solution at 37 °C, would be an appropriate system (Searl *et al*., 1999). [The Working Group noted that there is still some inter-laboratory variability in methodologies that affects the consistency of the dissolution rates reported.]

(ii) *Mechanisms of in-vitro fibre degradation*

The fibre dissolution model using the rate constant, k_{dis}, assumes simple (congruent) dissolution (all fibre components dissolve at approximately the same rate, which is constant over time), with fibre length and diameter decreasing at constant rates. In this model, the fibre composition does not change during the dissolution process. However, many MMVFs exhibit a high degree of leaching, i.e. incongruent dissolution, in which certain components dissolve more rapidly than others. Leaching of the fibres changes their composition over time and their morphologies show expanding zones of leached-out, lower-density material. This material has been reported to react with other components and form deposits on the fibres (Klingholz & Steinkopf, 1984; Leineweber, 1984; Potter & Mattson, 1991; Christensen *et al*., 1994; Hesterberg *et al*., 1996c; Bauer, 1998a,b; Hart *et al*., 1999). Differences in dissolution behaviour have been related to the chemical composition of the fibres, i.e. high contents of alumina and silica favour congruent dissolution, and to the manufacturing process (Potter & Mattson, 1991; Bauer, 1998a,b). In the manufacture of certain types of glass fibre, heating over a flame is used in the fiberization process, and has been associated with the formation of a resistant surface layer (Bauer, 1998b). Traditionally, fiberization in air at room temperature has been used in the manufacture of most standard thermal and acoustic insulation wools, while heating in a flame has been used for most special-purpose fibres (Hesterberg & Hart, 2001).

Durability is determined not only by dissolution of fibres in extracellular or intracellular fluid, but also by the physical condition of the fibre residuum; the fibre is no longer resident in the lung once it has disintegrated into non-fibrous particles (Scholze & Conradt, 1987). Furthermore, breakage of long fibres in the lung would increase the probability of their clearance by macrophages. A positive correlation between the leaching index (a measure of the change in fibre composition) and breakage was demonstrated in a study of the in-vitro dissolution of 18 compositions of glass fibre in Gamble's solution at pH 7.4. Fibre breakage *in vitro* was apparently caused by leaching-induced weakness followed by mild stress generated by the interwoven nature of the fibres in the test system (Hesterberg *et al*., 1996a; Bauer, 1998b). Striking fragmentation following in-vitro leaching was also exhibited by another rapidly-leaching MMVF, X-607 fibre (Hesterberg & Hart, 2001).

(iii) *Relationship between in-vivo retention of fibres, in-vitro dissolution rates and fibre composition*

A relationship between in-vivo retention of long fibres and in-vitro dissolution rates (k_{dis}) at near-neutral pH has been reported by Searl *et al.* (1999) and by Maxim *et al.* (1999a,b). In addition, Eastes *et al.* used linear regression techniques to develop equations for the estimation of the dissolution rate of a wide variety of MMVFs. The data used to develop these equations were derived from either data on in-vitro dissolution rates (Eastes *et al.*, 2000a), or from data obtained from in-vivo studies (Eastes *et al.*, 2000b,c). A correlation was shown between the estimate using these

equations and the actual values measured *in vitro* for a wide range of compositions of long fibres.

[The Working Group noted that the kinetic and thermodynamic approach used by Eastes *et al.* (2000a,b,c) derived from homogeneous physicochemical systems, may not fully apply to the complex multiphase system of a multicomponent fibre in a biological fluid, where leaching and redeposition processes also occur (Hesterberg *et al.*, 1996a).]

(iv) *Cellular in-vitro systems*

The effect of macrophages on the dissolution of long and short glass wool and rock (stone) wool fibres was studied in a flow-through cell-culture system. In this system, culture medium flowed through a membrane on which the macrophages and fibres were placed. Dissolution was estimated by measuring the concentrations of silicon, iron and aluminium in the culture medium after various time periods. Compared with the traditional static culture system, the flow-through system enables longer testing periods and avoids pH changes resulting in the build-up of dissolution products of cells and fibres (Luoto *et al.*, 1994a).

The dissolution of glass wool was faster in cell-free culture medium than in the presence of macrophages in cell culture. The dissolution of rock (stone) wool was slower than that of glass wool in cell culture. However, in a cell-free culture medium, the dissolution of experimental rock (stone) wool was more pronounced than that of commercial glass wool (Luoto *et al.*, 1995, 1998). Glass wool released silicon faster than rock (stone) wool, but rock (stone) wool released iron and aluminium faster than glass wool. Fibres in the flow-through culture exhibited surface changes, such as fractures, peeling and pitting. In the presence of macrophages, short fibres, < 20 μm in length, were easily phagocytosed whereas longer fibres were incompletely phagocytosed by a large number of alveolar macrophages. Macrophages that had engulfed fibres contained measurable amounts of silicon (Luoto *et al.*, 1998). Man-made vitreous fibres also induced ruffling and blebbing on the surface of rat alveolar macrophages: the exposed cells produced extensions which fastened them to the fibres or to other cells to form clumps or clusters of cells and fibres, each cell engulfing part of a fibre (Luoto *et al.*, 1994b).

Long, partly phagocytosed or non-phagocytosed glass wool fibres seemed to disappear more rapidly than short, phagocytosed glass wool fibres, in agreement with the observations of other investigators (Jaurand, 1994). However, it is possible that the number of long fibres decreases because they break into short fibres and not because they dissolve more rapidly than the short fibres. In general, the dissolution profiles obtained in the flow-through cell culture system were rather similar to those seen in the traditional static system, but the dissolution rate of individual fibres was more rapid in the former. The overall conclusion of these investigators was that cell-culture systems should be chosen rather than cell-free in-vitro systems when assessing fibre durability and dissolution (Luoto *et al.*, 1994a,b, 1995, 1996, 1998).

The dissolution in synthetic simulated lung fluid (Gamble's solution) of two different fibres, biosoluble HT (rock) stone wool and the traditional MMVF21 stone wool was investigated. Both fibres dissolved readily at pH 4.5, possibly due to the presence of organic acids that are able to form complexes with aluminium. This suggestion is supported by the observation that organic acids without this complex-forming ability were ineffective in stimulating dissolution. The HT (rock) stone wool dissolved faster than MMVF21, most probably because of the different ratios of Al/(Al+Si) in the two types of fibre (0.40 and 0.25, respectively) (Steenberg *et al.*, 2001). This ratio has been shown to correlate well with the dissolution rate at pH 4.5 of a range of fibres (Guldberg *et al.*, 2000).

(*b*) *In-vivo solubility*

Christensen *et al*. (1994) suggested that the length of an inhaled fibre may determine the pH environment in the lung to which that fibre is exposed — fibres longer than about 20 μm would be in the extracellular compartment where the pH is 7.4, while shorter fibres would tend to be in the intracellular compartment of macrophages at pH 4.5. Lehuédé *et al*. (1997) found that the changes in fibre composition observed *in vivo* were similar to those observed *in vitro* at pH 7.4, but not at pH 4.5, although only one type of fibre was tested at pH 4.5. See section 4.1.4(*a*)(iv) for a further discussion on fibre dissolution in in-vitro cell cultures of macrophages (Luoto *et al*., 1994a,b, 1995, 1998).

The retention of fibres in the lung was studied following the exposure of Fischer 344/N rats by inhalation and compared with dissolution in a cell-free flow-through system *in vitro*. The 10 samples of MMVF studied included compositions similar to commercial insulation materials as well as new glass fibres and rock (stone) wools developed to enhance solubility. The compositions of many of these fibres changed with increasing time in the lungs, with a depletion in sodium oxide, calcium oxide and magnesium oxide, and relative enrichment in silica and alumina (Lehuédé *et al*., 1997). The in-vivo findings agree with those reported by other investigators (Hesterberg *et al*., 1996a, 1998a,b).

Thus, if fibres are too long for macrophage-mediated clearance and if their chemical composition renders them resistant to breakdown in lung fluids (both intracellular and extracellular), they can be expected to accumulate during chronic exposure and remain in the lung and related tissues for considerable lengths of time. A recent study supports this hypothesis: Syrian golden hamsters were exposed for 18 months (6 h/day, 5 days/week) to comparable dusts of either standard glass fibre insulation or amosite asbestos. After one day of exposure, the lung burdens of longer fibres (> 20 μm in length) were similar for the two fibres (~0.2 million/lung), demonstrating comparable lung deposition for the two types of fibre. However, after 12 months of exposure, the lung burdens of the two types of fibre were different, i.e. 1×10^6 fibres (> 20 μm)/lung for glass wool, but 37×10^6 fibres (> 20 μm)/lung for amosite. Assuming little or no transport by macrophages of fibres > 20 μm in length, the almost 40-fold higher lung burden for amosite suggests that the fibres of the insulation glass wool dissolved or otherwise broke down

in lung fluid much more rapidly than those of amosite (Hesterberg *et al*., 1999; McConnell *et al*., 1999).

The chronic inhalation effects in rats of X-607 (a rapidly dissolving MMVF that does not fit into any of the traditional categories) were compared with those of RCF1. Fischer 344 rats were exposed to a fibre aerosol by nose-only inhalation for 6 h per day on five days per week for two years. The concentrations of the aerosols of X-607 and RCF1 were similar (~200 WHO fibres/cm^3) and the fibres had similar average dimensions (approximately 20 μm × 1 μm). The deposition of fibres in the lung after 6 h of inhalation was greater for X-607 than for RCF1. However, at all later times, the numbers of fibres per lung (especially fibres > 20 μm) were lower for X-607 than for RCF1, suggesting that X-607 was less biopersistent. Chemical analysis of fibres from the lung revealed more rapid leaching of X-607 than of RCF1; after 78 weeks in the lung, the calcium content of X-607 had decreased from an initial 40% (by mass) to zero, leaving a fibre of essentially pure silica. The chemical composition of RCF1 did not change. Fibres of X-607 also had a much higher in-vitro dissolution rate (k_{dis}, 990 ng/cm^2/h) than RCF1 (k_{dis}, 6 ng/cm^2/h; see below) (Hesterberg *et al*., 1998a).

Groups of Wistar rats were given a 50-mg dose of one of three MMVFs (glass wool, rock (stone) wool, glass microfibres (JM 100) by intraperitoneal injection. The chemical compositions of the fibres were analysed by inductively coupled plasma spectrometry carried out on the urine of the rats, which was collected at fixed times between day 1 and day 204, and analysed for elements known to be present in the original fibres. At day 204, a piece of omentum was removed at necropsy, ashed and analysed by energy dispersive X-ray diffraction analysis (EDXA) to identify the elements remaining in the fibres. Silicon and aluminium were found to be retained in the fibres from all samples at day 204. Titanium, present at 0.3% (w/w) in the original sample of rock (stone) wool, was not detectable by EDXA at day 204, but small quantitities were detected in urine collected during the first two weeks of exposure. The barium content of the retained glass microfibres (JM 100) had decreased by day 204 and barium was detectable in the urine. The authors concluded that titanium and barium could be suitable biomarkers of exposure to rock (stone) wool and glass microfibres and could also reflect the in-vivo dissolution of these fibres (Wastiaux *et al*., 1994). [The Working Group noted the preliminary nature of this study and the high dose used and also noted that not all rock (stone) wool contains titanium and barium.]

Baier *et al*. (2000) used instillation of MMVF10, RCF1a and HT fibre in rats as an in-vivo model to evaluate local biological responses to fibres of different composition. Specimens taken at 2, 7, 30 and 90 days post-instillation were compared by means of SEM and energy-dispersive X-ray spectroscopy (EDS). Fibres of MMVF10 dissolved the most quickly both in extracellular fluids and after phagocytosis; HT fibres were thinned by phagocytes and fragmented, and RCF1a resisted both external dissolution and uptake by macrophages, becoming embedded in granulomatous nodules. In accordance with these observations, the infrared spectra of sequentially-harvested slices of lung lobe showed increasing intensities of absorption bands associated with granuloma

formation only for RCF1a. These results indicated that the lung can process the fibres in different ways dependent on their composition.

Two studies have reported on the retention of fibres injected directly into the peritoneal cavity of rats.

Collier *et al*. (1994) reported that long glass fibres (> 20 μm) instilled into the lung of Fischer 344 rats underwent a reduction in diameter at a rate consistent with their exposure to a near-neutral pH environment. The same type of fibres, when injected into the peritoneal cavity, dissolved at a rate consistent with a more acidic environment. In a second study, Collier *et al*. (1995) compared the retention of fibres injected into the peritoneal cavity of Fischer 344 rats with the retention of the same fibres after intratracheal instillation into the lung. Long fibres (> 20 μm) were retained to a greater extent in the peritoneal cavity than in the lung. At doses in excess of 1.5 mg injected into the peritoneal cavity, most of the injected material was found in nodules which were either free in the peritoneal cavity or loosely bound to the peritoneal organs. The fibres tested had a mean diameter of 2.0 μm. [The Working Group noted that if biopersistence assays are conducted in serosal cavities, doses that do not induce a bolus effect should be chosen.]

4.2 Toxic effects in humans

4.2.1 *Adverse health effects other than respiratory cancer*

(*a*) *Mortality data*

Data covering all non-malignant respiratory diseases (NMRD) and the subgroup bronchitis, emphysema and asthma (BEA) associated with exposure to MMVFs are available from the death certificates of historical cohorts of MMVF production workers. Descriptions of the cohorts are given in section 2. The mortality data on NMRD are summarized in Table 66.

In the most recent update of the European cohort study, Sali *et al*. (1999) reported a non-significant excess of all NMRD among glass-wool workers (standardized mortality ratio (SMR), 1.18; 95% CI, 0.98–1.40). For the subgroup BEA, i.e. NMRD excluding influenza and pneumonia, the corresponding value was slightly lower (SMR, 1.12; 95% CI, 0.82–1.49). Interestingly, short-term MMVF workers (employed for < 1 year) in the study by Sali *et al*. (1999) of the European cohort (Boffetta *et al*., 1998) had significantly higher SMRs (SMR, 1.79 [95% CI, 1.8–2.44]) for BEA than long-term workers (employed for ≥ 1 year) (SMR, 0.95 [95% CI, 0.67–1.32]. [The Working Group noted that information on smoking history was not given in these studies.]

In the 1992 update of the US cohort of glass-fibre workers, the SMR for NMRD (excluding influenza and pneumonia) (national rates) was reported to vary according to the type of glass fibre produced, i.e. 0.80 for filament, 0.89 for wool and filament and 1.10 for mostly wool. These values were not significantly different from unity. The same pattern was observed using local rates (Marsh *et al*., 2001a). In the 1989 update of the

Table 66. Mortality data for non-malignant respiratory diseases (NMRD) in MMVF production workers and end-users

Reference	Study type	Country	No. of subjects	Industry	Relative risk (95% CI)	Observed	Comments
Glass (excluding continuous filament)							
Chiazze *et al*. (1992)	Nested case–control	USA	102	Production	Odds ratio, 1.5 (0.5–4.1)		Same odds ratio for fine fibres
Sali *et al*. (1999)	Cohort	Europe	5275	Production	SMR, 1.18 (0.98–1.40)	127	SMR (BEA), 1.12 (95% CI, 0.82–1.49)
Marsh *et al*. (2001a)	Cohort	USA	10 961	Production	SMR (all workers), 1.10 (0.91–1.32) SMR (long-term workers), 0.94 (0.72–1.20)	115 63	Production of mostly glass wool; SMR for NMRD excluding influenza and pneumonia
Rock (stone) and slag wool							
Sali *et al*. (1999)	Cohort	Europe	4616	Production	SMR, 0.97 (0.76–1.23)	71	SMR (BEA), 0.96 (95% CI, 0.66–1.35)
Marsh *et al*. (1996)	Cohort	USA	3035 N-cohort[a]	Production	SMR (LR), 1.07 NS	49	NMRD excluding influenza: SMR (LR), 1.27 (NS)
Marsh *et al*. (1996)	Cohort	USA	443 O-cohort[a]	Production	SMR (LR), 1.80 $p < 0.01$	29	NMRD excluding influenza: SMR (LR), 1.83 ($p < 0.05$)

Table 66 (contd)

Reference	Study type	Country	No. of subjects	Industry	Relative risk (95% CI)	Observed	Comments
Gustavsson *et al*. (1992)	Cohort	Sweden	2807	End-users (manufacture of prefabricated wooden houses)	SMR, 0.95 (0.65–1.35)	32 (33.6 expected)	Mixture of mineral wool insulation SMR (BEA), 0.98 (95% CI, 0.49–1.76)
Continuous glass filament							
Shannon *et al*. (1990)	Cohort	Canada	1465	Production	SMR (men), 0.43 [0.05–1.54][b] SMR (women), 1.62 [0.04–9.29][b]	2 men 1 woman	Based on a total of 96 deaths; SMR (LR) (BEA), 0.917 (95% CI, 0.479–2.842)
Chiazze *et al*. (1997)	Cohort	USA	2933 white men	Production	SMR (LR), 1.03 (0.68–1.48)	28 (27.28 expected)	Based on 437 deaths
Sali *et al*. (1999)	Cohort	Europe	1482	Production	1.05 (0.48–2.00)	9	SMR (BEA), 0.70 (95% CI, 0.14–2.03)
Marsh *et al*. (2001a)	Cohort	USA	5431	Production	SMR (all workers), 0.80 (0.61–1.03) SMR (long-term workers), 0.82 (0.59–1.12)	59 40	NMRD excluding influenza and pneumonia
Watkins *et al*. (1997)	Cohort	USA	1074 white women 494 black men	Production	SMR (LR) women, 0.82 (0.30–1.78) SMR (LR) men, 0.21 (0.005–1.17)	6 women 1 man	SMR (LR) (BEA) women, 1.18 (95% CI, 0.38–2.74) SMR (LR) (BEA) men, 0.38 (95% CI, 0.01–2.14)

BEA, bronchitis, emphysema, asthma; LR, local rate; NR, national rate; NS, not significant

[a] See section 2 for explanation of 'N-cohort' and 'O-cohort'

[b] Calculated by the Working Group

US cohort of rock (stone)/slag wool workers, the SMR (national rates) for NMRD (excluding influenza and pnemonia) in the five-plant group was 1.31 (95% CI, 0.93–1.79). Using local rates, the SMR was 1.27 (95% CI, 0.90–1.74). In another plant that had a documented history of asbestos use, the SMRs were statistically significant [2.07; 95% CI, 1.26–3.20; and 1.83; 95% CI, 1.12–2.83; using national and local rates, respectively] (Marsh *et al*., 1996). Although the US nested case–control study on cancer of the respiratory system suggested that all of the excesses in respiratory cancers may be explained by cigarette smoking, this has yet to be documented for NMRD excluding influenza and pneumonia. Moreover, smoking habits seem to differ slightly between the male workers in the glass fibre subgroup and in the rock (stone)/slag wool subgroup (Marsh *et al*., 2001b): this should be kept in mind when considering the mortality ratios for NMRD.

In a study of workers engaged in the manufacture of prefabricated wooden houses, no excess of NMRD was observed (SMR, 0.95; 95% CI, 0.65–1.35). However, this cohort had only a short follow-up period (Gustavsson *et al*., 1992).

(*b*) *Morbidity data*

(i) *Pneumoconiosis*

The data available on pneumoconiosis mainly come from radiological studies and are limited to standard thoracic X-rays that have low sensitivity and low specificity. No data are available from radiological examinations made by computerized tomography scanning. Recent studies are summarized in Table 67.

Most of the studies found no significant excess of small opacities on chest autoradiographs except the two studies of glass fibre production workers (Kilburn & Warshaw, 1991) and end-users (Kilburn *et al*., 1992). However, as stated by the authors, previous exposure to asbestos could have affected the observed results. Moreover, severe methodological limitations were reported (Rossiter, 1993; Weill & Hughes, 1996). [The Working Group acknowledged and agreed with the published comments concerning these limitations.]

Hughes *et al*. (1993) found no significant relation between small opacities observed in the chest autoradiographs of workers at seven MMVF production plants and exposure to MMVF as compared with controls. However, it should be noted that cumulative exposure to fibres was extremely low as stated in the initial publication by Weill *et al*. (1983) (less than 10 fibres–years/cm^3 in the group exposed to the finest fibres, < 1 μm in diameter; and less than 1 fibre–years/cm^3 in the group exposed to fibres > 3 μm in diameter). This study also demonstrated the notable variability of radiographic assessment. Table 68 summarizes the results of two consecutive readings from the two glass fibre plants where the highest level of exposure occurred (in the other three glass fibre plants, the cumulative level of exposure was estimated at less than 1 fibre–years/cm^3 (Weill *et al*., 1983)).

Table 67. Pneumoconiosis: radiographic data from MMVF production workers and end-users

Reference	Country	Industry	X-ray reading method	No. of exposed subjects	Percentage of abnormalities ≥ 1/0 among exposed subjects	No. of controls	Percentage of abnormalities ≥ 1/0 among controls	Comments
Glass (excluding continuous filament)								
Hughes *et al*. (1993)	USA	Production	5 ILO readers (median)	1252	1.8	272	0.7	NS
Kilburn & Warshaw (1991)	USA	Production	2 ILO readers (consensus)	175	12.6	–	–	Uninterpretable data (no control group; 137 subjects with previous exposure to asbestos)
Kilburn *et al*. (1992)	USA	End-users (insulation of refrigerators)	2 ILO readers (consensus)	284	8.1	–	–	Uninterpretable data (no control group; previous exposure to asbestos)
Rock (stone) and slag wool								
Hughes *et al*. (1993)	USA	Production	5 ILO readers (median)	183	0	33	0	
Yano & Karita (1998)	Japan	Production	1 ILO reader	440	0.5	544	0.2	No details for small opacities
Refractory ceramic fibre								
Lemasters *et al*. (1994)	USA	Production	3 ILO readers (median)	847	0	–	–	21 pleural plaques
Rossiter *et al*. (1994)	Europe	Production	3 ILO readers (median)	543	7	–	–	No relation with cumulative exposure to refractory ceramic fibres
Trethowan *et al*. (1995)	Europe	Production	[no details given]	592	13% ≥ 0/1 3.3% ≥ 1/1	– –	–	No relation with cumulative exposure to refractory ceramic fibres

Table 67 (contd)

Reference	Country	Industry	X-ray reading method	No. of exposed subjects	Percentage of abnormalities ≥ 1/0 among exposed subjects	No. of controls	Percentage of abnormalities ≥ 1/0 among controls	Comments
Lockey *et al*. (1996); Lawson *et al*. (2001)	USA	Production	3 ILO readers (median)	652	0.5	–	–	20 pleural plaques
Lockey *et al*. (2002)	USA	Production	3 ILO readers (median)	794	1.3	–	–	Non-significant excess risk with exposure to refractory ceramic fibres; significant relation with age and tobacco smoking
Cowie *et al*. (2001)	Europe	Production	3 ILO readers (median)	774	8	–	–	Non-significant excess risk with exposure to refractory ceramic fibres; significant relation with age and tobacco smoking; increase of opacities ≥ 1/0 (2%) on new reading of 1987 films

ILO, International Labour Organization; NS, not significant

Table 68. Autoradiographic assessments of small opacities in the lungs of workers in two glass fibre plants

	Cumulative exposure	No. of subjects	Percentage ≥ 1/0 1st radiographic assessment	Percentage ≥ 1/0 2nd radiographic assessment
Plant 3 (glass fibre diameter > 1 μm)	4.3 fibres–years/cm^3	220	5.9	3.2
Plant 5 (glass fibre diameter < 1 μm)	7.4 fibres–years/cm^3	122	6.6	0

From Hughes *et al.* (1993); data on cumulative exposure from Weill *et al.* (1983)

An Australian study reported morbidity data from 687 production workers in seven plants manufacturing both glass and rock (stone) wool. Small opacities (classified as 1/0 and 1/1) were detected on the chest radiographs of seven workers (only one ILO reader). However, no data on exposure were presented in the paper, and there was no control group (Brown *et al*., 1996). Similar findings were reported in a Japanese study of 493 male workers in nine rock (stone) wool production plants: the prevalence of radiographic abnormalities among 440 of these workers did not differ significantly from that in 544 control subjects or in a comparable population of 401 asbestos workers (Yano & Karita, 1998).

No data have been published concerning chest radiographs of production workers in the continuous glass filament industry.

Conflicting results were obtained on workers involved in the production of refractory ceramic fibres from the two most recent updates of the US and European cohorts. In the US study (Lockey *et al*., 2002), the incidence of irregular opacities (classified ≥ 1/0) showed a non-significantly elevated odds ratio for exposure to refractory ceramic fibres (workers with > 135 fibres–months/cm^3 cumulative exposure) (odds ratio, 4.7; 95% CI, 0.95–23.7). The same was observed for workers with > 10 years in refractory ceramic fibre production (odds ratio, 4.3; 95% CI, 0.9–28.3). As expected, small irregular opacities were significantly related to exposure to tobacco smoke and age. Similar results were observed in the European study (Cowie *et al*., 2001), which found an association between opacities (category ≥ 1/0) and exposure subdivided by calendar time period: a positive association was observed only with exposure that occurred before 1971, although this finding was based on few data (only 51 exposed workers).

(ii) *Pleural fibrosis and related findings*

Until now the non-malignant pleural effects of MMVFs in humans have been documented using only standardized thoracic radiography. As described for pneumoconiosis this technique has serious limitations: pleural plaques are correctly classified mainly when calcified; diffuse pleural thickening is well-recognized mainly when asso-

ciated with parenchymal bands. All other aspects are non-specific and can be related to other anatomical features. Moreover, other etiological factors having a definite association with pleural fibrosis are frequently present: mainly co-exposure to asbestos, but also the sequelae of infectious pleural diseases. Asbestos exposure is the most important confounding factor, since there is a strong co-linearity with exposure to MMVFs. Recent studies are summarized in Table 69.

The studies by Kilburn and Warshaw (1991) and Kilburn *et al*. (1992) reported the occurrence of pleural abnormalities in 7.4% and 7.0% of radiographs, respectively. [The Working Group noted that these studies are difficult to interpret for two main reasons: i.e. the absence of a control group and considerable exposure to asbestos which was not taken into account.]

In a study of rock (stone) wool production, Järvholm *et al*. (1995) reported a small, statistically nonsignificant, excess of circumscribed pleural thickening (pleural plaques). However, a nested case–control study failed to demonstrate a relationship with exposure to fibres (odds ratio for workers with ≥ 15 years of exposure, 1.2 [95% CI, 0.3–4.2]). [The Working Group noted that the members of this cohort were young; 57% of men and 86% of women were aged less than 40 years.] The data from the Australian (Brown *et al*., 1996) and Japanese (Yano & Karita, 1998) cohorts did not show any excess of pleural disease. [The Working Group noted that no excess of pleural abnormalities was observed in a comparison group of Japanese asbestos workers.]

A significant excess of pleural plaques was identified radiographically among a European cohort of refractory ceramic fibre production workers. The results presented in an initial report were inconclusive (Trethowan *et al*., 1995), since pleural anomalies did not show a dose–response. In contrast, the two publications of results from the US cohort (Lemasters *et al*., 1994; Lockey *et al*., 1996) demonstrated a significant relation with time since beginning of exposure and with cumulated duration of exposure. Moreover, the difference was still significant after adjustment for exposure to asbestos. The most recent updates of the cohorts in Europe and the USA contributed further information. The longitudinal data from the US cohort reinforced the initial results, showing a significant association (after adjustment for exposure to asbestos and age) with duration, latency and cumulative exposure (Lockey *et al*., 2002). For the highest classes of exposure duration (> 20 years), latency (> 20 years) and cumulative exposure (> 135 fibres/cm^3–months), the odds ratios were: 3.7 (95% CI, 1.1–11.8), 6.1 (95% CI, 1.9–27.1) and 6.0 (95% CI, 1.4–33.1), respectively. In the European cohort, the findings on pleural abnormalities were more difficult to interpret. Of a total of 774 subjects included in the update, 158 had been exposed to asbestos while employed in the refractory ceramic fibre industry and 351 (including 98 who were also exposed within the refractory ceramic fibre industry) were potentially exposed to asbestos outside the industry. The prevalences of pleural changes were lower among the 355 subjects not exposed to asbestos (Cowie *et al*., 2001) than the cohort as a whole (9% and 11% for pleural changes and 3% and 5% for pleural plaques, respectively). However, the 355 subjects not exposed to asbestos showed a significant association of pleural changes

Table 69. Pleural fibrosis: radiographic data from MMVF production workers and end-users

Reference	Country	Industry	X-ray reading method	No. of exposed subjects	Percentage of pleural abnormalities among exposed subjects	No. of controls	Percentage of pleural abnormalities among controls	Comments
Glass (excluding continuous filament)								
Kilburn & Warshaw (1991)	USA	Production	2 ILO readers (consensus)	175	7.4	–	–	Uninterpretable data (no control group; 137 subjects with previous exposure to asbestos)
Kilburn *et al.* (1992)	USA	End-users	2 ILO readers (consensus)	284	7.0	–	–	Uninterpretable data (no control group; previous exposure to asbestos)
Rock (stone) and slag wool								
Hughes *et al.* (1993)	USA	Production	5 ILO readers (median)	183	0	33	0	
Järvholm *et al.* (1995)	Sweden	Production	1 reader (radiophoto-graphy)	933	1.3% PP	865	0.3% PP	No relation with exposure to rock (stone) wool
Yano & Karita (1998)	Japan	Production	1 ILO reader	440	2.7	544	2.9	No details on pleural opacities
Refractory ceramic fibres								
Lemasters *et al.* (1994)	USA	Production	3 ILO readers (median)	686	3.1% PP 0.3 % DPT	–	–	Significant relation with exposure to refractory ceramic fibres (latency and duration) even after adjustment for exposure to asbestos
Rossiter *et al.* (1994)	Europe	Production	3 ILO readers (median)	543	2.7% PP or DPT; 0.9% bilateral	–	–	No relation with exposure to refractory ceramic fibres

Table 69 (contd)

Reference	Country	Industry	X-ray reading method	No. of exposed subjects	Percentage of pleural abnormalities among exposed subjects	No. of controls	Percentage of pleural abnormalities among controls	Comments
Refractory ceramic fibres (contd)								
Lockey *et al*. (1996)	USA	Production	3 ILO readers (median)	438	4.1% PP or DPT	214	0.9% PP or DPT	Significant relationship with exposure to refractory ceramic fibres (latency and duration) even after adjustment for exposure to asbestos
Lockey *et al*. (2002)	USA	Production	3 ILO readers (median)	794	3.4% any pleural abnormalities and 81% plaques	–	–	Significant relationship with duration, latency and cumulative exposure adjusted for exposure to asbestos and age
Cowie *et al*. (2001)	Europe	Production	3 ILO readers (median)	774	11% any pleural abnormalities and 5% plaques	–	–	Increased risk with time since first exposure to refractory ceramic fibres among 355 workers not exposed to asbestos

DPT, diffuse pleural thickening; ILO, International Labour Organization; PP, pleural plaque

with time since first exposure to refractory ceramic fibres, after adjustment for age, smoking habits and body mass index. When no adjustment was made for age, a highly significant association existed between time since first exposure to refractory ceramic fibres and both pleural changes and pleural plaques. [The Working Group considered it desirable to extend the survey of both cohorts with a tomodensitometric examination of the thorax for at least those subjects who showed pleural changes, since it is considered as a sensitive method to detect such abnormalities.]

(iii) *Symptoms and changes in lung function*

Data from morbidity studies have been collected using questionnaires and lung function tests. The results on symptoms are summarized in Table 70 and for lung function tests in Table 71.

Four studies have been published that concern mainly glass wool: two in the production industry and two among end-users. In the French study of 524 production workers, a significant increase in symptoms in the upper respiratory tract and of dyspnoea was noted. However, a nested case–control study did not confirm the occurrence of any impairment of lung function (Moulin *et al.*, 1987). The results of the USA production study were more complicated to interpret: asbestos was a major confounding factor since 137 out of 175 workers had been previously exposed to asbestos (Kilburn & Warshaw, 1991). Two studies of end-users, one of workers installing insulation in refrigerators (Kilburn *et al*., 1992) and the other of sheet-metal workers (Hunting & Welch, 1993) reported high rates of chronic bronchitis (10.9% and 15%, respectively). The interpretation of the results was hampered by the absence of a control group and the potential for co-exposure to air contaminants including asbestos. Moreover, the results from lung function tests did not suggest any dose–response relationship with fibre exposure.

Two studies of end-users of rock (stone) and slag wool or a mixture of mineral wools have been reported. Although an excess of symptoms in the upper respiratory tract and cough were reported from the first study in Denmark (Petersen & Sabroe, 1991) and a significant decrease of lung function (measured as 'forced expiratory volume') in the second Danish study (Clausen *et al*., 1993), other potential sources of occupational exposure were not taken into acount. Four studies of production workers have been conducted. In the study from France (Moulin *et al*., 1988), a high prevalence of symptoms (cough, sputum, symptoms in the upper respiratory tract) was reported to be associated with exposure to fibres: however, this result was observed in only one of the five plants investigated (a glass-wool plant where 51% of the study population worked), and where workers were also exposed to resin. A Danish study comparing 235 rock (stone) wool production workers with a random sample of 243 subjects from the general population (Hansen *et al*., 1999) demonstrated a significant incidence of airflow obstruction related to fibre exposure (14.5% in MMVF workers, 5.3% in controls), but this effect was limited to heavy smokers: the self-administered questionnaire on disease confirmed an excess of emphysema (9 cases, as against 2 in the control group) with a

Table 70. Chronic obstructive pulmonary diseases and other related findings in MMVF workers: results from questionnaires

Reference	Country	No. of subjects	Industry	Symptoms								Comment
				Upper airways	Cough	Sputum	Chronic bronchitis	Dyspnoea	Wheezing	Asthma	Relation with exposure	
Glass fibre (excluding continuous filament)												
Moulin *et al.* (1987)	France	524	Production	$p < 0.05$				$p < 0.05$			?	Inconsistency between plants; co-exposure to phenolic resins
Kilburn & Warshaw (1991)	USA	175	Production	1.1%[a], 19.4%[b]							?	Data uninterpretable (no control group; 137 subjects with previous exposure to asbestos)
Kilburn *et al.* (1992)	USA	284	End-users (insulation for refrigerators)	5.6%[a], 19.4%[b]			10.9%			6%	?	Data uninterpretable (no control group; previous exposure to asbestos)
Hunting & Welch (1993)	USA	333	End-users (sheet-metal workers)				15%				+	Other sources of exposure?
Glass and/or rock (stone) or slag wool												
Petersen & Sabroe (1991)	Denmark	2654	End-users (construction workers): glass wools	$p < 0.05$	$p < 0.05$						+	Other sources of exposure?
Moulin *et al.* (1988)	France	1839[c]	Production: glass wools	1.9–9.6%	6.9%–21.9%	4.3–9.2%		4.1–12%		0.9–1.4%	+	Co-exposure with phenolic resins
Hughes *et al.* (1993)	USA	1030	Production: fibrous glass and rock (stone) wools		13% (ns)		6% (ns)	5% (ns)		5% (ns)	–	

Table 70 (contd)

Reference	Country	No. of subjects	Industry	Symptoms								Comment
				Upper airways	Cough	Sputum	Chronic bronchitis	Dyspnoea	Whee-zing	Asthma	Relation with exposure	
Glass and/or rock (stone) or slag wool (contd)												
Brown *et al*. (1996)	Australia	533	Production		10.7%			12%	20.4%	7.3%	?	No data on exposure
Yano & Karita (1998)	Japan	493	Production: rock (stone) wool		1.4%	4.1%		0			–	No difference compared with a control group of urban residents
Hansen *et al*. (1999)	Denmark	377	Production: rock (stone) wool		26% (ns)	24% (ns)	3.8% (emphy-sema)	20% (ns)			+	Emphysema: 0.9% in a comparable control group
Refractory ceramic fibres												
Trethowan *et al*. (1995)	Europe	628	Production	55%	13%		12%		18%		+	Relation with dyspnoea significant
Cowie *et al*. (2001)	Europe	774	Production		24% (men) 20% (women)		3.3% (men) 2.4% (women)	3.2% (men) 8.5% (women)			?	Limited evidence of association with recent exposure to respirable fibres

ns, not significantly different from comparable control group

[a] Nose bleed and throat irritation

[b] Throat irritation

[c] Population of three plants with 1041, 535 and 263 participating workers; the percentages give the range of values.

Table 71. Chronic obstructive pulmonary diseases and other related findings from tests of lung function in MMVF workers

Reference	Type of study	Country	No. of subjects	Industry	Lung function (percentage of expected values)				Relationship with exposure	Comments
					TPC	FVC	FEV_1	$FEF_{25–75}$		
Glass fibre (excluding continuous filament)										
Kilburn & Warshaw (1991)	T	USA	175	Production	114.2	94.8	91.3	80.7	?	Uninterpretable data (no control group; 137 subjects with previous exposure to asbestos)
Kilburn *et al*. (1992)	T	USA	214[a]	End-users (insulation for refrigerators)	107.7	92.9	90.8	85.8	?	Uninterpretable data (no control group; previous exposure to asbestos)
Glass and rock (stone) and slag wool										
Hughes *et al*. (1993)	T	USA	1030	Production		103 (ns)	101.1 (ns)	78 (ns)	–	
Clausen *et al*. (1993)	L	Denmark	340	End-users (insulators)		ns	decreased ($p < 0.001$)		+	Other co-occurring exposure
Brown *et al*. (1996)	T	Australia	687	Production	ns	93 (ns)	95 (ns)		–	No data on exposure
Rock (stone) wool										
Hansen *et al*. (1999)	T	Denmark	235			96 (ns)	92 (ns)		+	Elevated risk only in heavy smokers
					TPC	FVC	FEV_1	$FEF_{25–75}$		

Table 71 (contd)

Reference	Type of study	Country	No. of subjects	Industry	Lung function (percentage of expected values)				Relationship with exposure	Comments
					TPC	FVC	FEV_1	$FEF_{25–75}$		
Refractory ceramic fibres										
Burge *et al*. (1995); Trethowan *et al*. (1995)	T	Europe	628	Production		110.6	104.6	84.1	+	Significant relation (FEV_1) with exposure seen only among smokers
Lemasters *et al*. (1998)	L	USA	736	Production		$p < 0.01$ for male smokers; $p < 0.05$ for female non-smokers	$p < 0.01$ (male smokers)		+	Sex-specific respiratory effect
Lockey *et al*. (1998)	L	USA	361	Production		reduced ($p < 0.05$)	reduced ($p < 0.05$)		–	Longitudinal follow-up, with selection bias due to lower level of recent exposure
Cowie *et al*. (2001)	L	Europe	774	Production		reduced ($p < 0.05$)	reduced ($p < 0.05$)		+	Significant effects limited to male current smokers

T, transversal; L, longitudinal; ns, not significant; TPC, total pulmonary capacity; FVC, forced vital capacity: FEV_1, forced expiratory volume in 1 s; $FEF_{25–75}$, forced expiratory flow (mid-flow)

[a] Workers without pulmonary opacities

relative risk of 4.5 (95% CI, 1.0– 20.6). The absence of any alteration in single-breath carbon monoxide-diffusion capacity in this population was interpreted by the authors as absence of lung fibrosis. [The Working Group noted that chest radiographs were not available in this study. In the studies from America (Hughes *et al*., 1993), Japan (Yano & Karita, 1998) and Australia (Brown *et al*., 1996), the results (assessed by questionnaire and/or tests of lung function) showed no association with exposure.]

In contrast, the studies of the American and European cohorts of refractory ceramic fibre workers did report statistically significant numbers of abnormalities related to exposure to fibres. In the American cohort (Lemasters *et al*., 1998), the relative risk of having at least one respiratory symptom was 2.9 (95% CI, 1.4–6.2) for men and 2.4 (95% CI, 1.1–5.3) for women. In male workers, a significant loss of forced vital capacity (FVC) of the lung noted in workers exposed to refractory ceramic fibres was limited to current and ex-smokers. The decline in FEV_1 (forced expiratory volume in 1 s) was significant only in current smokers. A significant loss of FVC was observed among female non-smokers. The initial findings of the European study (Burge *et al*., 1995; Trethowan *et al*., 1995) also demonstrated obstruction of the airways related to cumulative exposure to refractory ceramic fibres that was limited to current and ex-smokers. In the most recent update of this cohort (Cowie *et al*., 2001), a significant negative association between estimated cumulative exposure to refractory ceramic fibres and lung function (FVC and FEV_1) was found. [The Working Group noted that the results of this study should be reassessed since there was no indication of pulmonary interstitial disease (no alteration in single-breath carbon monoxide-diffusion capacity and an absence of small parenchymal opacities on chest radiographs), and total lung capacity was not monitored in this study.]

(*c*) *Other respiratory findings*

Isolated case reports on lung proteinosis (1 case) and lung fibrosis (2 cases) have been published (Takahashi *et al*., 1996; Riboldi *et al*., 1999; Yamaya *et al*., 2000). [The Working Group noted the lack of an association with occupational exposure to fibres in these studies.]

In a series of 50 consecutive cases of sarcoidosis, twelve patients were described whose exposure to MMVFs had been assessed either by job history or by mineralogical lung analysis (Drent *et al*., 2000a,b). [The Working group noted that the information on exposure history and the mineralogical data from analysis of lung samples were incomplete.]

Only one study was intended to test an initial event of pulmonary fibrosis through determination of serum type III procollagen aminoterminal propeptide. In this series the concentration of this propeptide in 56 male workers exposed to rock (stone) wool during production (0.05–0.75 fibres/cm^3 for up to 20 years) did not differ from that of a group of 20 controls (Cavalleri *et al*., 1992).

4.2.2 *Other toxic effects*

(*a*) *Mortality data*

Data on other non-malignant, non-respiratory causes of death after exposure to MMVFs are available from the US and European cohorts. In the 1985 follow-up of the US cohort, Marsh *et al.* (1990) noted a slight increase in the incidences of nephritis and nephrosis for the whole cohort (SMR (national rate), 1.46 ($p < 0.01$)). The US rock (stone) and slag wool cohort had an elevated risk for nephritis and nephrosis (N-cohort SMR (local rate), 2.02; $p < 0.05$; O-cohort SMR (local rate), 2.58; not significant; see section 2) and no risk for cirrhosis of the liver (N-cohort SMR (local rate), 0.90; O-cohort SMR (local rate), 0.91) (Marsh *et al*., 1996). A case–control study of workers at one plant from the US cohort reported no association between exposure to glass fibre and nephritis, nephrosis or cirrhosis of the liver (Chiazze *et al*., 1999). The corresponding SMRs for the total US cohort of glass fibre workers (Marsh *et al*., 2001a) were 1.04 (local rate) [95% CI, 0.81–1.32] and 0.88 (local rate) [95% CI, 0.75–1.04], respectively. In the European cohort, an excess of cirrhosis was observed only among the continuous filament production workers (SMR (national rate), 2.12 [95% CI, 1.10–3.71] for 12 observed cases) (Sali *et al*., 1999).

(*b*) *Morbidity data*

Most of the literature concerning the irritating effects of MMVFs on the skin and mucosa was published before the last IARC evaluation (IARC, 1988). More recent publications have reported on observations in contaminated buildings (Lockey & Ross, 1994; Thriene *et al*., 1996; Bergamaschi *et al*., 1997): the role of fibres with diameter > 4 μm and direct contact with deposited fibres was emphasized.

Recent epidemiological studies are scarce. In a plant manufacturing and processing mineral wool for insulation, 25% of 259 workers presented with a skin disease, resulting from an allergy related to MMVF additives (Kiec-Swierczynska & Szymczk, 1995). Dermal irritation is a common symptom of the sick-building syndrome. Forty-six of 103 white collar workers in a building in which the atmosphere was contaminated by mineral fibres from ceiling panels had symptoms of dermal irritation (Thriene *et al*., 1996). Similarly, it was reported that 32% of 66 subjects investigated for sick-building syndrome demonstrated positive patch tests with mineral fibres (Thestrup-Pedersen *et al*., 1990). Among 2654 Danish construction workers, a statistically significant increase in the frequency of symptoms of irritation of the skin, eyes and respiratory tract were reported (Petersen & Sabroe, 1991).

4.3 Toxic effects in experimental systems

This section covers selected toxic effects of fibres in experimental systems that are believed to be potentially important in relation to the carcinogenic process. These endpoints include in-vivo effects such as inflammation and fibrosis, as well as selected in-

vitro assessments including cytotoxicity, oxidant production and alterations to the cell cycle including proliferation and apoptosis. Genetic toxicology end-points are reviewed in section 4.5.

It is important to appreciate the degree to which biopersistence plays a role in the different studies and end-points under review, as this property of fibres is thought to be critical in determining chronic toxicity and carcinogenic outcome in humans and in experimental animal systems. In-vitro assays are invariably short-term (i.e. from hours to days), and the effect of fibre durability is unlikely to be detected in such assays. [The Working Group noted that endotoxin is a potent environmental contaminant and its presence in fibre samples could enhance their ability to cause acute inflammation. The presence of endotoxin or the steps taken to inactivate it, were not always reported.] Therefore, short-term tests could give a misleading impression of possible long-term biological effects. This will most likely become manifest as a false-positive result in an in-vitro assay for long, non-biopersistent fibres. For a non-biopersistent fibre, the effects seen *in vitro* may apply only to the time interval *in vivo* before the fibre begins to undergo dissolution or breakage. In contrast, a durable fibre may show the effects much more slowly and is more likely to give rise to pathological change.

4.3.1 *Continuous glass filament and glass wool*

Pathological change related to inflammation and fibrosis in rat lungs exposed to fibres is commonly quantified by the Wagner scale (see Table 72). The original Wagner scale was modified by McConnell *et al*. (1984). Wagner scores have been given here as the score preceded by the abbreviation Wag, e.g. Wag 4. Pathological effects might be expected to occur in rats with scores of Wag 3 and higher, while the presence of fibrotic lesions is identified by scores of Wag 4 and higher.

Table 72. Wagner pathology grading scale[a]

		Cellular change
Normal	1	No lesion
Minimal	2	Macrophage response
Mild	3	Bronchiolization, inflammation
		Fibrosis
Minimal	4	Minimal
Mild	5	Linking of fibrosis
Moderate	6	Consolidation
Severe	7	Marked fibrosis and consolidation
	8	Complete obstruction of most airways

From Hesterberg *et al.* (1993)

[a] According to guidelines given by McConnell *et al.* (1984)

(*a*) *Inflammation and fibrosis*

Hesterberg *et al*. (1996b) exposed Fischer 344 rats for 6 h per day, on five days per week, for 13 weeks to MMVF10 at the following airborne mass concentrations (exposure by inhalation is generally expressed as the airborne mass concentration followed by the fibre concentrations in parentheses): 3.2 mg/m^3 (36 WHO fibres/cm^3, 14 fibres > 20 μm long/cm^3), 16.5 mg/m^3 (206 WHO fibres/cm^3, 81 fibres > 20 μm long/cm^3), 30.5 mg/m^3 (316 WHO fibres/cm^3, 135 fibres > 20 μm long/cm^3), 44.5 mg/m^3 (552 WHO fibres/cm^3, 223 fibres > 20 μm long/cm^3) and 62.2 mg/m^3 (714 WHO fibres/cm^3, 343 fibres > 20 μm long/cm^3). Inflammation, measured as increased amounts of polymorphonuclear neutrophils and protein in bronchoalveolar lavage, was produced by exposure to MMVF10 at concentrations of 16 mg/m^3 and above. An influx of alveolar macrophages in the bronchioles and alveoli was seen at the end of the study in all dose groups.

Fischer 344 rats treated with RCF1 and positive controls treated with chrysotile asbestos were compared with rats treated for 6 h per day, on five days per week for 24 months, with glass fibre types MMVF10 and MMVF11 at the following airborne mass concentrations: MMVF10, 3.1 mg/m^3 (29 WHO fibres/cm^3), 17.1 mg/m^3 (145 WHO fibres/cm^3) and 27.8 mg/m^3 (232 WHO fibres/cm^3); MMVF11, 4.8 mg/m^3 (41 WHO fibres/cm^3), 15.8 mg/m^3 (153 WHO fibres/cm^3) and 28.3 mg/m^3 (246 WHO fibres/cm^3). Positive control rats were exposed to chrysotile at 10.1 mg/m^3 (10 600 WHO fibres/cm^3) and rats treated with RCF1 received 29.1 mg/m^3 (187 WHO fibres/cm^3). Animals exposed to chrysotile had scores of Wag 4 after three months of exposure; the scores remained at the same level for up to 24 months of continued exposure; there was no decrease over up to 18 months of post-treatment recovery. Rats exposed to RCF1 (30 mg/m^3) did not reach a score of Wag 4 until six months after the start of treatment; the scores remained at Wag 4 for the following 18 months of exposure and during the period of post-exposure recovery. There were clear time- and exposure-dependent responses in the pathological scores for MMVF10 and MMVF11, which peaked at Wag 3 later in rats exposed to 16 mg/m^3 and earlier after exposure to 30 mg/m^3. There was clear evidence of a decrease in the Wag score during recovery after short-term exposure, but not when the duration of exposure (to MMVF10) had been ≥ 18 months (Hesterberg *et al*., 1993).

Hesterberg *et al*. (1998a) exposed Fischer 344 rats by inhalation for 6 h per day on five days per week for up to two years to one of the following: the relatively-soluble glass fibre X-607 at 30 mg/m^3 (174 WHO fibres/cm^3, 47 fibres > 20 μm long/cm^3); RCF1 at 29 mg/m^3 (187 WHO fibres/cm^3, 101 fibres > 20 μm long/cm^3); and chrysotile at 10 mg/m^3 (10 600 WHO fibres/cm^3; 0 fibre > 20 μm long/cm^3). The Wagner scores were as follows: controls exposed to clean air, Wag 1; treatment with X-607, average Wag 2.5; treatment with RCF1 and chrysotile, average Wag 4.0. In animals allowed to recover after cessation of exposure, the Wag score decreased in the rats exposed to X-607, but not in the animals exposed to RCF1. Pathological indices demonstrated the

absence of bronchoalveolar or pleural collagen after treatment with X-607, but varying scores of up to Wag 2.7 after exposure to RCF1 and chrysotile. These scores generally decreased during periods of post-exposure recovery. The data suggest that soluble fibres are less pathogenic than more persistent fibres and that their pathogenic effects are reversible to some extent.

In the study by Everitt *et al*. (1994), Fischer 344 rats were exposed by intrapleural injection to RCF1 at 0.56×10^6 WHO fibres/cm^3 or to MMVF10 at 10.5×10^6 WHO fibres/cm^3. The preparation of RCF1 contained 1.75×10^6 particles (fibres with length/diameter < 3)/cm^3, the MMVF10 sample contained 1.17×10^6 particles/cm^3. At 28 days post-exposure there was little difference between the two groups in mediastinal weight or pleural thickness. [This should be viewed against the much greater exposure to MMVF10 on a per fibre basis.]

Drew *et al*. (1987) examined the effects in Fischer 344 rats of 10 repeated intratracheal instillations (1 h/week for 10 weeks; 0.5 mg per treatment) of specially made and sized glass fibres obtained from the Thermal Insulation Manufacturers' Association (TIMA). The average fibre dimensions were 1.5×5 μm or 1.5×60 μm. Another group of rats was treated with unsized UICC (Union internationale contre le Cancer [International Union Against Cancer]) crocidolite. The lesions seen after the 10 weekly instillations of glass fibres were described as aggregations of macrophages and foreign-body granulomas; no striking differences were observed between the results of the treatment with long and short glass fibres. Rats given five weekly instillations of crocidolite asbestos showed not only macrophage responses, but also accumulation of haemosiderin within the macrophages and thickening and fibrosis of the alveolar wall. [The Working Group noted that these pathological changes are consistent with a bolus effect due to instillation of a large quantity of foreign material.]

Cullen *et al*. (2000) studied the pathogenicity of E-glass fibres (code 104/E) and microfibres (code 100/475), with amosite asbestos as a control. Wistar rats were exposed by inhalation for 7 h per day on five days per week for one year to E-glass (1022 WHO fibres/cm^3, 72 fibres > 20 μm long/cm^3), microfibres (code 100/475) (1119 WHO fibres/cm^3, 38 fibres > 20 μm long/cm^3) and amosite (981 WHO fibres/cm^3, 89 fibres > 20 μm long/cm^3). One year after the end of exposure, the mean level of advanced fibrosis (percentage of lung area affected, rather than Wagner score) was determined. The results were as follows: controls, 0.08; treatment with E-glass, 8.0; treatment with microfibres, 0.2; and exposure to amosite, 7.6. The authors attributed their findings to the higher number of long fibres of E-glass and their greater biopersistence compared with that of other fibre types.

Syrian golden hamsters received MMVF10a by nose-only inhalation for 6 h per day, on five days per week for 13, 26 and 52 weeks at an airborne mass concentration of 30 mg/m^3 (323 WHO fibres/cm^3, 151 fibres > 20 μm long/cm^3), while MMVF33 (475 glass wool), a special-purpose fibre that is more durable than MMVF10a, was delivered at a concentration of 37 mg/m^3 (283 WHO fibres/cm^3, 106 fibres > 20 μm long/cm^3). Other groups of animals were exposed to amosite at three concentrations:

0.8 mg/m^3 (33 WHO fibres/cm^3, 9 fibres > 20 μm long/cm^3), 3.7 mg/m^3 (157 WHO fibres/cm^3, 37 fibres > 20 μm long/cm^3) and 7.3 mg/m^3 (255 WHO fibres/cm^3, 67 fibres > 20 μm long/cm^3). The Wagner pathology scores showed time-dependent increases for all types of exposure tested. At 52 weeks of exposure, the scores were as follows: controls, Wag 0; treatment with MMVF10a, Wag 2.3; treatment with MMVF33, Wag, 4.0; and treatment with amosite (at 0.8 mg/m^3), Wag 4.0. Exposure to the two higher concentrations of amosite produced the following Wag scores: amosite at 3.7 mg/m^3, Wag 5.3; amosite at 7.3 mg/m^3, Wag 6.0. The authors concluded that there was a clear effect of increased durability causing a greater pathological response, and that the pathological effects could be related to the numbers of long fibres (> 20 μm) persisting in the lungs (Hesterberg *et al*., 1997).

Hesterberg *et al*. (1999) exposed Syrian golden hamsters by inhalation to MMVF10.1 (a thinner fibre than MMVF10) and MMVF10a (a batch of MMVF10 that had a slightly lower fluorine content and was slightly thinner than MMVF10) for 6 h per day, on five days per week for 13 weeks with or without a period of 10 weeks of post-exposure recovery. The airborne mass concentrations of MMVF10.1 used ranged from 3.2–62.2 mg/m^3 (62.2 mg/m^3 contained 714 WHO fibres/cm^3; 343 fibres > 20 μm long/cm^3). The pathology score never exceeded Wag 2 for any exposure group and decreased to Wag 1 after recovery following the lower levels of exposure. Bronchoalveolar lavage analysis revealed that only exposure to airborne concentrations ≥ 16.5 mg/m^3 (206 WHO fibres/cm^3, 81 fibres > 20 μm long/cm^3) caused inflammation, as judged by a significant increase in the number of neutrophils.

In a chronic study by McConnell *et al*. (1999), Syrian golden hamsters were exposed for 6 h per day, on five days per week for 78 weeks to MMVF10a at 29.6 mg/m^3 (339 WHO fibres/cm^3, 134 fibres > 20 μm long/cm^3), MMVF33, a more durable special-purpose glass, at 37.0 mg/m^3 (310 WHO fibres/cm^3, 109 fibres > 20 μm long/cm^3) or amosite at three concentrations (highest dose, 7.1 mg/m^3, 263 WHO fibres/cm^3, 69 fibres > 20 μm/cm^3). Both visceral and parietal fibrosis were described. Visceral pleural fibrosis (expressed as the fibrosis index, i.e. the area of polarizable pleural collagen per length of pleural basement membrane, mm^2/mm) was estimated at 78 weeks in the periphery of the entire lobes and averaged 3.1 in the controls, 3.83 in hamsters exposed to MMVF10a, 3.97 in hamsters exposed to MMVF33 (these values are not significantly different from those of the controls) and 8.86 ($p < 0.05$) in hamsters exposed to the high dose of amosite. After six weeks of recovery, these figures showed a decrease in animals exposed to MMVF10a, but a further increase in the animals exposed to MMVF33 and to the high dose of amosite.

Rutten *et al*. (1994) injected Fischer 344 rats and Syrian golden hamsters intra-pleurally (injection volumes, 1.4 mL and 1.0 mL, respectively) with RCF1 (6–312 μg) or MMVF10 (43–2000 μg); MMVF10 contained about three times more WHO fibres than RCF1. The objective was to have the same number of long fibres in both groups. There was little difference between the effects of these two treatments in rats, but hamsters had more thickening of the visceral pleura (fibrosis) after treatment with

MMVF10; in contrast, more proliferation of mesothelial cells was observed in both the visceral and parietal pleura in hamsters treated with RCF1.

Donaldson *et al*. (1995a) exposed rats by inhalation for 7 h to 1000 WHO fibres/cm^3 long-fibre amosite asbestos or to the more soluble code 100/475 glass fibres which had comparable length distributions, and assessed cell proliferation by means of incorporation of bromodeoxyuridine 24 h later. Increased cell proliferation was seen throughout the lung after exposure to amosite, but not after exposure to code 100/475 glass fibres. This result after short-term exposure suggests that effects other than solubility, which was unlikely to be a factor, can determine the short-term proliferative response to inhaled fibres; a difference in surface reactivity between the fibres used is a possible explanation.

Donaldson *et al*. (1993) used a number of fibre types from the repository of the TIMA to assess the ability of the fibres to cause short-term inflammation in the peritoneal cavity of the C57BL6 mouse, when equal numbers of each type of fibre were used. Inflammation was not related to the mass instilled, which varied greatly depending on the type of fibre tested. A similar degree of inflammation was caused by MMVF10, MMVF11, MMVF21 and MMVF22; UICC crocidolite was most inflammogenic, probably reflecting its greater content of long fibres. These data highlight the limited usefulness of short-term assays in discriminating between fibres of variable biopersistence.

(*b*) *Cell toxicity*

The results of many studies on short-term cytotoxic effects have been published. The reports evaluated in this section have been chosen according to their ability to meet the following criteria:

- The dose was expressed as number of fibres given, in the way that exposure to fibres is regulated, and allowing potency to be compared.
- Fibre length was specified so that false-negative results from preparations of short fibres could be excluded.
- Adequate documentation of the fibre source was supplied to allow cross-reference to longer-term studies and to define composition.
- Studies involving instillation of fibres directly into the lungs were screened to exclude those in which an excessive dose might confound the results (overload effects).
- Where possible, control fibres were included or different categories of fibre length were used.

Hart *et al*. (1994) used Chinese hamster ovary cells, which are not direct target cells, to assess the cytostatic effects of glass fibre MvL 901 (Schuller International, Denver, CO) of various lengths and thicknesses at equal fibre number. Cell proliferation was inhibited to approximately 25% of control values by longer fibres (average length, 25 μm), whereas short fibres (average length, 3.5 μm) did not inhibit cell proliferation. Fibre thickness (tested with MvL 475) had a modest effect, with

thinner fibres (0.3 μm) being more effective inhibitors of cell proliferation than thicker fibres (7 μm) at equal fibre number. This study showed that long fibres are toxic *per se*, in addition to their ability to accumulate because of their slower clearance from the lung. [The Working Group noted that, on a number basis, longer fibres also have a larger surface area than shorter fibres.]

Code 100 glass fibres were separated by dielectrophoresis into five mean length categories: 33, 17, 7, 4 and 3 μm. In general, the longer fibres were thicker; the average diameter was 0.75 μm for fibres in the longest category versus 0.35 μm in the shortest category. Four doses of fibres with equal fibre numbers for each length category were tested for their ability to inhibit lucigenin chemiluminescence and to cause release of lactate dehydrogenase from rat alveolar macrophages. The fibres showed length-related toxicity; the 17-μm- and 33-μm-long fibres had a similar high potency, and fibres ≤ 7 μm long had a significantly lower potency (Blake *et al*., 1998). The authors noted that their study pointed to a direct cellular basis for the increased toxicity of long fibres, namely frustrated phagocytosis, leading to leakage of oxidants and enzymes from a macrophage that is trying to engulf a fibre.

(*c*) *Cell activation*

Brown *et al*. (1998a) tested a series of fibres that included MMVF10 and code 100/475 for their ability to activate release of superoxide anions by rat alveolar macrophages at equal WHO fibre number. Uncoated (naked) fibres of both types inhibited superoxide release, but after coating with IgG the MMVF10 fibres, but not the code 100/475 fibres, showed stimulation. Amosite and RCF1 showed a similar pattern of response. Thus, there was no discrimination in this assay between fibres defined as either pathogenic or non-pathogenic by some studies of chronic toxicity and oncogenicity in animals (see secton 3).

Two subsets of the size-separated code 100 glass fibres used by Blake *et al*. (1998) with average lengths of 17 and 7 μm, respectively, were tested in cultured RAW 264.7 mouse macrophage cells. The production of tumour necrosis factor α and the activation of the transcription factor were more strongly induced by 17-μm-long fibres than by 7-μm-long fibres on a per fibre basis (Ye *et al*., 1999).

(*d*) *Other effects*

Brown *et al*. (2000b) examined the ability of equal numbers of WHO fibres (MMVF10, MMVF11 and JM code 100/475) to deplete antioxidants from solution in comparison with refractory ceramic fibres and amosite. The ability to deplete antioxidants did not correlate with the pathogenic or non-pathogenic properties of fibres (as defined by some studies of their chronic toxicity and oncogenicity in animals). The MMVF10 and code 100/475 fibres were more active in depleting glutathione and vitamin C than amosite asbestos.

Jensen and Watson (1999) used the SV-40-immortalized human mesothelial cell line MeT-5A and the rhesus monkey kidney epithelial cell line LLC-MK_2 to test the ability of MMVF10a, crocidolite, chrysotile and RCF1 (0.3 or 1.0 mg/mL) to impair separation of cells at the end of mitosis (cytokinesis). By use of high-resolution time-lapse microscopy, all fibres longer than about 15 μm, regardless of composition, were seen to be trapped in the cleavage furrow of the dividing cells. This caused failure of cytokinesis resulting in the formation of multinucleate cells.

Gilmour *et al*. (1995) tested the ability of MMVF10 and MMVF11 at equal numbers of WHO fibres to cause oxidative cleavage of bacterial plasmid supercoiled φX174RF_1 DNA. The MMVF10 and MMVF11 fibres showed little or no activity when compared with amosite and crocidolite asbestos, and this was not related to the ability of the fibres to release bioavailable iron.

Fisher *et al*. (1998) measured the release of bioavailable (soluble) iron from various types of man-made fibres (MMVF10, code 100/475, RCF1 and silicon carbide) and from long-fibre amosite asbestos. When equal numbers of WHO fibres were tested, more iron was released by MMVF10 than by code 100/475, which released about the same amount of iron as amosite; generally, more iron was released under acid conditions (pH 4.5) than under neutral conditions (pH 7.2) and there was more release into surfactant than into saline. The authors concluded that simple quantification of the release of soluble (bioavailable) iron from fibres under these in-vitro conditions cannot discriminate between pathogenic and non-pathogenic fibres.

Zoller and Zeller (2000) tested a sample of a respirable glass wool fibre (code A) and a sample of rock (stone) wool (code G) to examine whether fibres lost their biological activity when incubated in unbuffered saline. Both untreated fibres and fibres that had been incubated for four weeks in saline solution were tested for their ability to affect the production of reactive oxygen species by cultured human HL-60 cells, as assessed by luminol-enhanced chemiluminescence. Incubation in saline solution reduced the ability of these glass wool and rock (stone) wool fibres to cause chemiluminescence mediated by reactive oxygen species, suggesting that leaching or shortening *in vivo* might also occur, decreasing the long-term reactivity of the fibre. These effects were not seen with the rock (stone) wool fibres MMVF21 and HT-N.

4.3.2 *Rock (stone) and slag wool*

(*a*) *Inflammation and fibrosis*

Creutzenberg *et al*. (1997) exposed Wistar rats by inhalation for one or three weeks to MMVF21 at 38.7 mg/m^3 (695 WHO fibres/cm^3) or 43.8 mg/m^3 (879 WHO fibres/cm^3), or for three weeks to RCF1 (51.2 mg/m^3, 679 WHO fibres/cm^3); there were similar proportions of long fibres in the aerosol clouds of both fibre types. Following a three-day recovery period, inflammation, as measured by increased numbers of polymorphonuclear leukocytes in bronchoalveolar lavage, was observed after treatment with both 38.7 mg/m^3 and 43.8 mg/m^3 of MMVF21: 4.7% and 13.4%, respectively, compared

with 1.1% in the lavage of rats exposed to clean air. In contrast, the rats exposed to refractory ceramic fibres had 18.9% polymorphonuclear leukocytes. During the extended post-exposure recovery period, the levels of polymorphonuclear leukocytes in the rats exposed to MMVF21 had returned to those of control animals by three months (0.5% and 0.8% in the groups treated with 38.7 mg/m^3 and 43.8 mg/m^3, respectively); however, at this time-point, the rats treated with RCF1 still had 13.5% polymorphonuclear leukocytes, although by 12 months this had decreased to 3.4%, which was not different from controls.

McConnell *et al*. (1994) exposed Fischer 344/N rats for 6 h per day, on five days per week for up to 24 months to MMVF21 at 3.1 mg/m^3 (34 WHO fibres/cm^3, 13 fibres > 20 μm long/cm^3), 16.1 mg/m^3 (150 WHO fibres/cm^3, 74 fibres > 20 μm long/cm^3) and 30.4 mg/m^3 (243 WHO fibres/cm^3, 114 fibres > 20 μm long/cm^3). Other groups of rats were exposed to MMVF22 at 3.1 mg/m^3 (30 WHO fibres/cm^3, 10 fibres > 20 μm long/cm^3), 16.1 mg/m^3 (131 WHO fibres/cm^3, 50 fibres > 20 μm long/cm^3) or 29.9 mg/m^3 (213 WHO fibres/cm^3, 99 fibres > 20 μm long/cm^3). Crocidolite (exposure period, 10 months; dose, 10 mg/m^3; 1610 WHO fibres/cm^3; 236 fibres > 20 μm long/cm^3) was used as a positive control. After various times up to 24 months, lung pathology was assessed and expressed as Wagner scores. The negative controls scored Wag 1 and animals exposed to crocidolite had a score of Wag 4 from three months of exposure onwards. Exposure to 16.1 mg/m^3 and 30.4 mg/m^3 MMVF21 resulted in scores of Wag 4 after 18 months which did not decrease after a period of recovery. Animals that received 29.9 mg/m^3 MMVF22 scored Wag 3 from six months onwards, and these scores decreased to Wag 2 or Wag 2.5 during recovery.

Kamstrup *et al*. (1998) exposed Fischer 344 rats to MMVF21 at a concentration of 16.1 mg/m^3 (150 WHO fibres/cm^3, 74 fibres > 20 μm long/cm^3) or 30.4 mg/m^3 (243 WHO fibres/cm^3, 114 fibres > 20 μm long/cm^3) or MMVF34 (soluble HT rock (stone) wool), at 30.5 mg/m^3 (288 WHO fibres/cm^3, 86 fibres > 20 μm long/cm^3) for 6 h per day, on five days per week for up to 18 months. At this time, the Wagner pathology score was Wag 4 in rats exposed to both the 16.1 mg/m^3 and 30.4 mg/m^3 doses of MMVF21 and 2.8 for rats exposed to MMVF34. The authors concluded that the lower biopersistence of the MMVF34 provided the most likely explanation for the observed differences. The same study reported the percentage of lung parenchyma affected with fibrosis or showing interstitial collagen in a morphometric assay (Kamstrup *et al*., 2001). A maximum of 0.5% collagen deposition (expressed as percentage of lung parenchyma affected) was reported for rats exposed to MMVF34 at 24 months, whilst in rats exposed to MMVF21 the scores were 1.68% after exposure to 16.1 mg/m^3 and 3.84% after exposure to 30.4 mg/m^3 at the same time-point. The authors concluded that this study provided further evidence that biopersistence is important in the pathogenicity of fibres.

Donaldson (1994) attempted to adapt short-term inflammation assays in the mouse peritoneal cavity to detect differences between fibres of variable biopersistence by including a pre-treatment step in which the fibres were incubated for four weeks at neutral or mild acid pH. Pre-treatment of MMVF21 and RCF1 fibres at pH 5.0 decreased

the degree of inflammation caused by MMVF21, but not RCF1 when compared with the effect seen after pre-treatment at pH 7.0. The author considered these findings confirmed that MMVF21 is not biopersistent at pH 5.0 and either shortens or dissolves. [The Working Group noted that the treated fibres were not sized or counted to confirm this contention.]

(*b*) *Cell activation*

Hill *et al*. (1996) assessed the ability of rat alveolar macrophages to release superoxide anions in response to treatment with uncoated or immunoglobulin (IgG)-coated MMVF21, code 100/475, RCF1 and silicon carbide fibres at equal numbers of WHO fibres. There was little release of superoxide in response to uncoated fibres of any type but, after incubation with IgG, the ability of both MMVF21 and RCF1 to cause superoxide release was enhanced. This effect appeared to be related to the high affinity of these fibres for the IgG, which is present in lung lining fluid and could also be adsorbed to the fibres *in vivo* to cause this enhancing effect.

Zoller and Zeller (2000) assessed the production of reactive oxygen species (ROS) by cultured human HL-60 cells using three types of rock (stone) wool fibre: code G, MMVF21 and HT-N (origin of the fibres specified in the paper). All samples were respirable and had the following proportions of long fibres: code G, 34% > 15 μm; MMVF21, 10% > 5 μm; HT-N, 50% > 15 μm. Both the untreated fibres and fibres that had been incubated at 37 °C in unbuffered saline solution for four weeks were tested. Pre-incubation reduced the ability of the code G rock (stone) wool fibres to cause chemiluminescence mediated by reactive oxygen species, but had no effect on MMVF21 or HT-N fibres.

(*c*) *Other effects*

Gilmour *et al*. (1995) assessed the ability of MMVF21 and MMVF22 at equal numbers of fibres to cause scission of supercoiled plasmid DNA in a cell-free system. These two fibres had little or none of this activity when compared with amosite asbestos. Tests on a series of fibres revealed no relationship between the ability of a fibre to release bioavailable iron and its pathogenicity.

4.3.3 *Refractory ceramic fibres*

(*a*) *Inflammation*

Refractory ceramic (aluminosilicate) fibres have been studied *in vivo* in rats and in Syrian golden hamsters and were reported to induce inflammation in the lung following inhalation.

In long-term inhalation studies (McConnell *et al*., 1995), Syrian golden hamsters were exposed for 18 months by nose-only inhalation for 6 h per day on five days per week to ‘size-selected’ kaolin-based RCF1 at an airborne mass concentration of

~30 mg/m^3 (approximately 220 fibres/cm^3; diameter, 1 μm; length, 25 μm). Inflammation of the lung was noted histologically as macrophage infiltration and microgranuloma formation. This study was designed to assess carcinogenicity (see section 3).

In chronic inhalation studies similar to those described above, the same kaolin-based RCF1 fibre preparation together with three other refractory ceramic fibres, *viz* an aluminium zirconium silica fibre (RCF2), a high-purity fibre (RCF3), and an 'after-service' heated kaolin-based fibre (RCF4) (all preparations contained an appreciable number of non-fibrous particles), were administered to Fischer 344 rats by nose-only inhalation (Mast *et al*., 1995b). The animals were exposed for 6 h per day, on five days per week for 24 months to the size-selected (1 μm diameter and ~20 μm length; 220 fibres/cm^3) refractory ceramic fibres at an airborne concentration of ~30 mg/m^3. Inflammation of the lung was noted histologically as macrophage infiltration and microgranuloma formation and was found to be treatment-related in all groups exposed to refractory ceramic fibres beginning at three months of exposure. In a follow-up dose–response study of RCF1, Mast *et al*. (1995a) used the same exposure regimen for airborne fibre concentrations of 3, 9 or 16 mg/m^3 (~36, 91 and 162 fibres/cm^3, with equal or larger numbers of non-fibrous particles (≤ 3 μm) and observed mild macrophage infiltration and microgranuloma formation by 12 months in rats in all concentration groups. The carcinogenicity data from this study have been reviewed in section 3.

In a re-analysis of the above-mentioned experiments, the authors of more recent studies of well-characterized RCF1 and RCF1a fibres, containing 25% and 2% non-fibrous particles, respectively, concluded that the non-fibrous particulate fraction of the aerosol used in these chronic studies was likely to be partially responsible for the persistent inflammation that resulted in the long-term effects in the lungs of rats exposed to the 30-mg/m^3 concentration (Brown *et al*., 2000a; Mast *et al*., 2000b).

Analysis of bronchoalveolar lavage fluid provided cytological and biochemical evidence that subchronic inhalation of RCF1 fibres causes pulmonary inflammation. Creutzenberg *et al*. (1997) exposed rats for three weeks to RCF1 at a single concentration of 51.2 mg/m^3 (679 WHO fibres/cm^3) and noted increased numbers of polymorphonuclear leukocytes for up to three months following cessation of exposure. Gelzleichter *et al*. (1999) conducted a 12-week inhalation study (treatment for 4 h per day, on 5 days/week) in both Fischer 344 rats and Syrian golden hamsters with a single RCF1 concentration of ~46 mg/m^3 (~300 WHO fibres/cm^3, with about 32% non-fibrous particles) and found cytological and biochemical indications of inflammation in bronchoalveolar lavage and pleural fluid in both species.

(*b*) *Fibrosis*

In the long-term studies mentioned above (Mast *et al*., 1995b; McConnell *et al*., 1995), RCF1 fibres were demonstrated to be fibrogenic in Fischer 344 rats and Syrian golden hamsters. When both species were exposed long-term by nose-only inhalation for 6 h per day on five days per week to RCF1 at an airborne mass concentration of ~30 mg/m^3, with approximately 220 fibres/cm^3 (size-selected fibres: 1 μm in diameter

and ~25 μm in length), both rats and Syrian golden hamsters were shown by histological analysis to have deposits of collagen at the bronchiolo–alveolar duct junctions. In addition to pulmonary parenchymal fibrosis, rats and hamsters exposed to RCF1 had pleural fibrosis (McConnell *et al*., 1995). In hamsters chronically exposed to RCF1, pulmonary fibrosis was less widespread than in control animals treated with chrysotile while pleural fibrosis was more marked.

In the long-term inhalation study of refractory ceramic fibres (Mast *et al*., 1995b), rats were exposed to RCF1, RCF2, RCF3 and RCF4 fibres. Fibrotic changes in the lungs developed after six months of exposure to RCF1 and RCF3. Collagen deposition was noted by histopathology after 24 months of exposure in response to all four preparations of refractory ceramic fibres. In addition, minimal to mild multifocal deposition of collagen was noted in the pleura of rats exposed to RCF1, RCF2 and RCF3 as well as in positive control animals exposed to chrysotile asbestos.

The long-term studies described above suggest that Syrian golden hamsters are more predisposed to the pleural effects of inhaled RCF1 than Fischer 344 rats. This finding is supported by other studies (Everitt *et al*., 1997; Gelzleichter *et al*., 1999) in which Fischer 344 rats and Syrian golden hamsters were exposed under the same experimental conditions, namely by nose-only inhalation to RCF1 for 4 h per day on five days per week at a single concentration (~46 mg/m^3, ~300 WHO fibres/cm^3) for 12 weeks. At 12 weeks post-exposure, the hamsters, but not the rats, had significant increases in deposition of pleural collagen, which correlated with an increased pleural mesothelial reaction, as assessed by cell proliferation.

(*c*) *Cell proliferation*

Rutten *et al*. (1994) demonstrated proliferation of mesothelial cells seven and 28 days after direct intrapleural instillation of RCF1 into Fischer 344 rats and Syrian golden hamsters. Hamsters demonstrated more extensive proliferation of mesothelial cells than did rats exposed to similar numbers of MMVF10 glass or RCF1 refractory ceramic fibres: RCF1 fibres induced a higher rate of proliferation in both species than MMVF10 glass fibres.

More recent studies by Gelzleichter *et al*. (1999) involving exposure by inhalation of both Fischer 344 rats and Syrian golden hamsters to a single concentration of RCF1 (~46 mg/m^3, ~300 WHO fibres/cm^3) for 12 weeks demonstrated proliferation of visceral and parietal mesothelial cells. Stronger and more persistent effects were seen in the hamsters than in the rats. [The Working Group noted that this study employed only one exposure concentration and did not compare RCF1 with any other fibrous particulates.]

(*d*) *Cellular toxicity*

Dopp *et al*. (1995) tested asbestos (chrysotile, amosite and crocidolite) fibres and refractory ceramic fibres [type unspecified] for their ability to induce apoptosis in cultured Syrian hamster embryo cells. Treatment with 0.5–10 μg/cm^2 refractory ceramic fibre or amosite for 16–72 h showed a small (3–3.4%), but not dose-related, deve-

lopment of apoptotic nuclei. This is in contrast to a significantly higher rate of apoptosis observed in Syrian hamster embryo cells treated with chrysotile (5 μg/cm^2) (up to 33%). [The Working Group noted that the dose levels, as expressed in number of fibres, were different.]

Hart *et al*. (1994) used Chinese Hamster ovary cells to assess the toxic effects of a large variety of fibres of various lengths and thicknesses, including crocidolite, chrysotile and various synthetic vitreous fibres. The primary effect of all the compositions tested was a concentration-dependent decrease in cell proliferation as a result of the physical disruption of cell division. These effects were strongly correlated with fibre length, but not with diameter. The effects did not differ between fibre types, which led the authors to conclude that this in-vitro system could not discriminate between fibres of different pathogenicity.

Refractory ceramic fibre [type unspecified] was found to be cytotoxic to cultured rat alveolar macrophages (24 h of exposure to 100, 300, or 1000 μg/mL), as assessed by the release of lactate dehydrogenase, but this effect was small in comparison with that observed after exposure to silica at a similar mass dose (Leikauf *et al*., 1995). Fujino *et al*. (1995) treated rat alveolar macrophages with refractory ceramic fibres [type unspecified] (12.5–200 μg/mL) and determined the release of tumour necrosis factor, lactate dehydrogenase and β-glucuronidase. In comparison with a series of natural mineral and synthetic vitreous fibres, the refractory ceramic fibres exhibited relatively low levels of biological activity. This was in contrast to the findings of earlier studies on an aluminium silicate fibre (Nadeau *et al*., 1987) which under certain experimental conditions elicited a greater release of lactate dehydrogenase and β-glucuronidase from macrophages than did chrysotile. [The Working Group noted that differences in fibre type, preparations, dimensions and dose may explain these results.]

(*e*) *Cell activation*

Refractory ceramic fibres have been demonstrated to activate polymorphonuclear neutrophils through an increase in intracellular calcium (Ruotsalainen *et al*., 1999). In this study, the production of reactive oxygen species induced by refractory ceramic fibres [not specified] in polymorphonuclear neutrophils was markedly higher than with other synthetic vitreous fibres, although the study did not use fibres of equivalent length distribution.

Treadwell *et al*. (1996) compared the abilities of RCF1 and asbestos to cause adherence of neutrophils to monolayers of endothelial cells at non-cytotoxic concentrations. Crocidolite and chrysotile asbestos, but not RCF1, activated endothelial cells and induced adherence of neutrophils through the expression of endothelial adhesion molecules such as intercellular adhesion molecule-1. In similar studies Barchowsky *et al*. (1997) found that crocidolite and chrysotile, but not RCF1, activated tyrosine kinases, increased cell motility and caused marked changes in endothelial cell morphology.

(*f*) *Oxidant generation*

Brown *et al*. (1998b) compared MMVF10, code 100/475, RCF1, RCF4, silicon carbide and amosite asbestos for their ability to generate free radicals using two assays: the assay of DNA φX174 plasmid scission and a salicylate assay of hydroxyl radical activity. In the plasmid assay, only long-fibre amosite asbestos produced free radical activity, while in the salicylate assay, both RCF1 and amosite caused release of hydroxyl radicals.

Hill *et al*. (1996) assessed the ability of rat alveolar macrophages to release superoxide anions in response to treatment with uncoated and immunoglobulin (IgG)-coated synthetic vitreous fibres. The studies used equal numbers of fibres of MMVF21, code 100/475, RCF1 and silicon carbide. There was little release of superoxide in response to uncoated fibres of any type but, after incubation with IgG, the activities of both MMVF21 and RCF1 fibres were enhanced in this respect. This appeared to be related to the high affinity of these fibres for the IgG, which is present in lung lining fluid and which could be adsorbed *in vivo* to cause this enhancing effect.

(*g*) *Other effects*

Brown *et al*. (2000b) examined the ability of equal numbers of fibres of different types to deplete antioxidants from lung lining fluid and from solutions of individual antioxidants. Included in the series of MMVFs tested were RCF1 and RCF4. The ability to deplete antioxidants did not correlate with differences between pathogenic and non-pathogenic fibres (as defined in long-term rodent bioassays). In comparison with MMVF10 glass fibres, RCF1 and RCF4 were not found to be especially active in depleting glutathione in these studies.

4.4 Effects on gene expression

4.4.1 *Continuous glass filament and glass wool*

Driscoll (1996) reviewed the role of inflammatory cytokines, growth factors and proto-oncogenes in asbestos-induced carcinogenesis. Churg *et al*. (2000) highlighted the role of reactive oxygen species in fibre-induced activation of signalling pathways controlling the expression of pro-inflammatory cytokines and growth factors.

In a sub-chronic inhalation study, the exposure of male Syrian golden hamsters to 901 glass fibre (MMVF10.1) at a concentration of ≥ 16 mg/m^3 for 6 h per day on five days per week for 13 weeks resulted in proliferation of epithelial cells (measured by immunostaining of proliferating cell nuclear antigen) and inflammation at bifurcations of the bronchoalveolar duct (Hesterberg *et al*., 1999). However, the staining intensity returned to normal after a 10-week recovery period and the proliferative response to glass fibres was lower than that for inhalation of amosite. This difference in the magnitude and duration of the proliferative activity of amosite asbestos in comparison with glass fibre (code 100/475) was also demonstrated by the use of bromodeoxy-

uridine-labelling in male Wistar rats 16 h after a single 7-h period of exposure to fibres (Donaldson *et al*., 1995a). The authors concluded that glass fibres on a mass basis may be less potent activators of cell proliferation *in vivo* than amosite asbestos.

The fibre-induced expression of DNA damage-inducible genes, proto-oncogenes or tumour suppressor genes has recently been investigated. Johnson and Jaramillo (1997) reported that, unlike crocidolite, code 100 glass microfibres failed to enhance the expression of DNA damage-inducible genes (*p53*, *Cip 1* and *Gadd 153*) in an alveolar epithelial type II tumour cell line (A549).

Exposure to MMVF10 at 25 μg/cm^2 did increase the concentrations of mRNA for c-*fos* and c-*jun* in rat pleural mesothelial cells, but it was less potent than crocidolite as it affected these proto-oncogene mRNA levels only at doses which caused cytotoxicity. High concentrations of MMVF10 also increased mRNA levels for ornithine decarboxylase in hamster tracheal epithelial cells, indicating induction of cell proliferation (Janssen *et al*., 1994).

The mammalian activator protein-1 family comprises homodimers and heterodimers of the Jun, Fos and ATF (activating transcription factor) subgroups of transcription factors (Shaulian & Karin, 2001). Activator protein-1 regulates the expression and function of regulators of the cell cycle, such as cyclin D1, p53, p21$^{Cip1/Waf1}$, p19^{ARF} and p16 and, therefore, plays an important role in cell proliferation. Mitogen-activated protein kinases (MAPK) play an important role in the signalling pathway for activator protein-1: extracellular signal-regulated kinase (ERK) can activate c-*fos* while Jun N-terminal kinase (JNK) activates c-*jun* and ATF (Karin, 1995). An increase of c-Jun protein has been shown in BALB/c-3T3 cells transformed by AAA-10 glass fibres (Gao *et al*., 1997). In addition, exposure of rat alveolar macrophages to code 100 glass fibres induced the phosphorylation of p38 and ERK MAPK (Ye *et al*., 2001). Exposure to code 100 glass fibres also stimulated the DNA binding activity of activator protein-1. Activation of activator protein-1 was partially inhibited by SB203580 (an inhibitor of p38) or PD98059 (which prevents the phosphorylation of ERK), indicating a role for MAPK in the signalling pathway to activator protein-1. Glass fibres (MMVF10) have also been shown to activate activator protein-1 in rat alveolar macrophages, but the strength of this effect was only about a third of that observed for amosite (Gilmour *et al*., 1997).

Tumour necrosis factor α (TNF-α) is an inflammatory mediator that has also been implicated to play a role in cell proliferation and apoptosis. Treatment of rat tracheal epithelial explants with TNF-α has been shown to enhance the binding of MMVF10 fibres to the cell surface. This enhancement was mediated through the activation of nuclear factor-κB (NF-κB) (Xie *et al*., 2000). Indeed, binding of TNF-α to the TNF type 1 receptor has been linked to phosphorylation of JNK which results in activation of activator protein-1 and cell proliferation (Karin & Delhase, 1998). Brass *et al*. (1999) have reported that the fibroproliferative response at the junctions of the bronchiolar–alveolar duct induced by inhalation of chrysotile is directly related to fibre-induced expression of mRNA and protein for TNF-α and transforming growth factor β1

(TGF-β_1); C57BL/6 mice were found to be susceptible to development of this lesion while 129 inbred mice were resistant.

Since TNF-α has been linked to fibre binding and cell proliferation, it is important to determine the effect of glass fibres on its production. To date, conflicting results have been reported. Cullen *et al*. (1997) showed that, unlike amosite or crocidolite, glass fibres (MMVF10 and MMVF11) failed to induce TNF-α production in cultures of rat alveolar macrophages. Similarly, Code 100/475 or MMVF10 glass fibres failed to significantly stimulate the release of TNF-α from rat alveolar macrophages, human blood monocytes, THP-1 human macrophage cell line or J774.2 mouse macrophage cell line in culture (Fisher *et al*., 2000). Similarly, glass fibres [not further specified] did not significantly stimulate TNF-α production by J774 cells (Murata-Kamiya *et al*., 1997). In contrast, significant stimulation by glass fibres [not further specified] of the release of TNF-α from rat alveolar macrophages was reported by Fujino *et al*. (1995), with a potency that was only slightly less than that of crocidolite, chrysotile or amosite asbestos.

A significant increase in TNF-α production was reported after in-vitro exposure for 6 h and 16 h to code 100 glass fibres (size-selected by dielectrophoresis) at a fibre:cell ratio of 5:1 in the mouse peritoneal monocyte cell line RAW 264.7 and in NR 8383 rat alveolar macrophages. Glass fibres 17 μm long were found to be two to four times more potent than short fibres (7 μm) in stimulating release of TNF-α at equal fibre numbers. Code 100 microfibres also activated the TNF-α gene promoter in RAW 264.7 monocyte and NR8383 alveolar macrophage cell lines; the 17-μm-long fibres were two to three times more potent than the 7-μm fibres. Several transcription factors have been identified as being involved in the expression of TNF-α induced by glass fibres. That is, the release of TNF-α in response to exposure to glass fibres was almost completely inhibited by an NF-κB inhibitor (SN50), by 70% with an inhibitor of p38 (SB203580) and by about 50% with an inhibitor of ERK (PD98059), which suggested the involvement of both NF-κB and activator protein-1. Similarly, fibre-induced TNF-α gene promoter activity was also inhibited by blockers of NF-κB and mitogen-activated protein kinase phosphorylation. In addition, transfection of NR8383 rat alveolar macrophage cells with an inactivated cAMP response element inhibited the glass-fibre-induced activation of the TNF-α gene promoter by about 80% (Ye *et al*., 1999, 2001).

In addition to the proto-oncogenes discussed above, increased cell proliferation has been linked to the secretion of inflammatory cytokines and growth factors from lung cells exposed to asbestos (Driscoll, 1996). The transcription factor NFκB is involved in the activation pathway for several such cytokines and growth factors induced by various particles and fibres (Schins & Donaldson, 2000). Brown *et al*. (1999) reported that amosite, the refractory ceramic fibre RCF1 and silicon carbide fibres were effective stimulants of NF-κB activation in lung type II epithelial cells (A549 tumour cell line). In contrast, twice the number of fibres of MMVF10 were required to activate NF-κB while code 100/475 glass fibres were inactive even at this high dose. Similarly, Gilmour *et al*. (1997) showed that amosite asbestos activated

NF-κB in rat alveolar macrophages *in vitro*, but MMVF10 failed to cause a significant activation of this transcription factor. In contrast, in-vitro treatment for 2 h of RAW 264.7 mouse monocytes with code 100 glass fibres at a fibre:cell ratio of 5:1 caused a substantial increase in the DNA-binding activity of NF-κB; the 17-μm fibres caused a threefold increase and the 7-μm fibres a twofold increase. The inhibition of the translocation of NF-κB from the cytoplasm to the nucleus by the NF-κB inhibitor (SN50) virtually eliminated the TNF-α production induced by glass fibres. There appears to be a positive feedback loop between NF-κB and TNF-α. Not only does activation of NF-κB stimulate the TNF-α gene promoter and TNF-α production (Ye *et al*., 1999), but TNF-α has been shown to cause a 10-fold increase in NF-κB activity in rat lung epithelial alveolar type II cells and to deplete the concentrations of IκB-α, an endogenous inhibitor of NF-κB activity (Janssen-Heininger *et al*., 1999).

As stated above, NF-κB has been implicated in the upregulation of several pro-inflammatory cytokines and growth factors observed after exposure to various particles and fibres (Schins & Donaldson, 2000). Driscoll *et al*. (1996) reported that in-vitro exposure of rat lung type II epithelial cells to crocidolite for 6 h increased mRNA concentrations for chemokines, macrophage inflammatory protein-2 and cytokine-induced neutrophil chemoattractant, but, under similar conditions, MMVF10 glass fibres did not. In an immunofluorescence assay, neither MMVF10 nor crocidolite asbestos fibres (length, < 60 μm) affected epidermal growth factor–receptor protein staining intensity and distribution in an SV-40-immortalized human mesothelial cell line (Met5A) (Pache *et al*., 1998), whereas crocidolite fibres > 60 μm in length increased the staining intensity and altered the distribution of this protein. It was noted by the authors that the MMVF10 glass fibres used in this study were only 20 μm in length, which may explain their ineffectiveness. [The Working Group noted that fibres in this length category usually do show effects in other cellular assays.] Glass fibres [not specified] reduced the plating efficiency of B14F28 Chinese hamster fibroblasts at concentrations > 2 μg/cm^2, but increased colony formation at concentrations < 0.33 μg/cm^2 (Fischer *et al*., 1998). This proliferative effect appears to be due to fibre-induced production of growth factors by these cells, since conditioned medium (supernatant medium of fibre-treated colonies) was also effective in stimulating colony growth. Glass fibres were somewhat less potent than amosite or crocidolite asbestos in this assay. In contrast, Koshi *et al.* (1991) observed an inhibition of colony formation in Chinese hamster lung cells exposed to code 100, code 104, code 108A and code 108B fibres, with TD_{50} values (concentrations required for 50% inhibition) of 10, 11, 18 and 27 μg/mL, respectively.

Fibres and particles have been reported to activate signalling pathways for NF-κB and activator protein-1 (Ding *et al*., 1999; Schins & Donaldson, 2000). Fibre-induced generation of reactive oxygen species has been implied from the results of a φX174 RF plasmid DNA assay to detect oxidant-induced DNA damage (Gilmour *et al*., 1995; Donaldson *et al*., 1996). In this assay, amosite and crocidolite asbestos were shown to generate significant amounts of reactive species in solution, whereas glass fibres (MMVF10 and MMVF11) were virtually ineffective. In contrast, code 100 glass fibres

and glass wool (Owens Corning) generated reactive species (hydroxyl radicals) in solutions containing hydrogen peroxide at rates higher than those observed with crocidolite and amosite asbestos, as detected by a salicylate assay (Maples & Johnson, 1992). Code 100/475 and MMVF10 glass fibres were also able to decrease the concentrations of glutathione and ascorbate in cell-free solutions, suggesting that glass fibres can generate reactive oxygen species under these conditions (Brown *et al*., 2000b). Similarly, in-vitro treatment with MMVF10 decreased the concentrations of glutathione in rat alveolar macrophages, although to a lesser extent than treatment with amosite asbestos (Gilmour *et al*., 1997). Wang *et al*. (1999a) reported that glass fibres were effective in stimulating the release of superoxide anions from guinea-pig alveolar macrophages *in vitro*, their potency being about 70% of that of chrysotile. However, glass fibres and chrysotile asbestos were equally potent in stimulating hydrogen peroxide production in these macrophages. This level of oxidant production was associated with a depletion of glutathione in these phagocytes, but glass fibres were not as effective as chrysotile in this respect. Superoxide release was also observed to occur in rat and hamster alveolar macrophages exposed *in vitro* to code 100 glass microfibres, or to MMVF21 coated with IgG (Hansen & Mossman, 1987; Mossman & Sesko, 1990; Hill *et al*., 1996). By means of chemoluminescence detection, the release of reactive oxygen species was measured in human polymorphonuclear neutrophils exposed *in vitro* to MMVF10, MMVF11, MMVF22, various types of refractory ceramic fibre or glass wool and in human monocytes exposed to MG-1 microglass fibres (Leandersen & Tagesson, 1992; Luoto *et al*., 1997; Ruotsalainen *et al*., 1999; Ohyama *et al*., 2000).

Oxidant production is believed to play an important role in mediating the proliferative response of lung cells to fibres. Churg (1996) reported that antioxidants decreased the uptake of asbestos fibres by explants of rat trachea. Antioxidants such as curcumin and pyrrolidine dithiocarbamate have been shown to inhibit the amosite-induced translocation of NF-κB to the nucleus in the lung epithelial tumour cell line A549 while aspirin inhibited crocidolite-induced activation of activator protein-1 in the mouse JB6 epidermal cell line (Brown *et al*., 1999; Ding *et al*., 1999). Aspirin was also shown to be effective in inhibiting crocidolite-induced activation of activator protein-1 in the bronchiolar tissue of mice, when given 30 min before intratracheal instillation of the fibres (Ding *et al*., 1999). In addition, exposure of rat lung epithelial alveolar type II cells to 1 mM 3-morpholinosydnonimine (which releases nitric oxide and superoxide which then react to yield peroxynitrite) caused an eightfold increase in activity of NF-κB. This oxidant-induced activation of NF-κB can be enhanced synergistically by TNF-α (Janssen-Heininger *et al*., 1999). Ye *et al*. (1999) have also shown that oxidants play a role in the activation of NF-κB in mouse monocytes (RAW 264.7 cell line) exposed *in vitro* to code 100 glass fibres (length, 17 μm). Treatment with *N*-acetyl-L-cysteine (a non-specific antioxidant) completely inhibited glass fibre-induced activation of NF-κB, activation of the TNF-α gene promoter and TNF-α production.

Increases in free intracellular calcium have been associated with cell proliferation through induction of c-*fos* and activation of activator protein-1 (Karin *et al*., 1997).

Glass wool (not specified) has been reported to increase the intracellular calcium concentration in human polymorphonuclear neutrophils exposed *in vitro* (Ruotsalainen *et al*., 1999). Similarly, microglass fibres (not specified) mobilized intracellular calcium in guinea-pig alveolar macrophages *in vitro* (Wang *et al*., 1999a). In both these studies, the potency of glass fibres to increase free intracellular calcium was found to be significantly lower than that of refractory ceramic fibres or chrysotile.

4.4.2 *Rock (stone) wool, slag wool and refractory ceramic fibres*

(*a*) *Pulmonary epithelial cells*

Proto-oncogene (*c-fos*, *c-jun*) mRNA levels were studied *in vitro* in hamster tracheal epithelial cells exposed to 5–25 μg/cm^2 crocidolite, MMVF10 or RCF1 (Janssen *et al*., 1994). Changes in *c-fos* expression were not detected after exposure to any of these three fibre types. Upregulation of *c-jun* was detected in response to crocidolite, MMVF10 and RCF1, but the increase caused by MMVF10 and RCF1 was less pronounced than with crocidolite.

Reactive oxygen and nitrogen species mediate cell proliferation and the production of pro-inflammatory cytokines through signal transduction and transcription factor activation. Brown *et al*. (1999) exposed A549 human lung epithelial type II tumour cells *in vitro* to equal numbers of fibres of long-fibre amosite, silicon carbide, RCF1 and RCF4 (8.24×10^6 fibres/mL) and investigated the induction of nuclear translocation of NF-κB. After exposure to amosite, silicon carbide or RCF1 the translocation of NF-κB to the nucleus was increased; a similar effect was observed after exposure to MMVF10 at 16.5×10^6 fibres/mL. No increase was detected after exposure to RCF4. Nuclear translocation of NF-κB was inhibited by antioxidants, such as curcumin.

Marks-Konczalik *et al*. (1998) exposed SV-40-immortalized human bronchial epithelial (BEAS 2B) cells to crocidolite, rock (stone) wool (115-4) and silica at concentrations of 0–50 μg/cm^2 and measured manganese superoxide dismutase gene expression and activity. At high concentrations (above 25 μg/cm^2), a decrease in the activity of manganese superoxide dismutase was seen in response to crocidolite and silica, but not to rock (stone) wool. Upregulation was seen in cells exposed to low concentrations (2 μg/cm^2) of all three fibre types. The decreased activity of manganese superoxide dismutase coincided with increased cytotoxicity.

Ljungman *et al*. (1994) exposed rat alveolar macrophages to equal masses of a range of mineral fibres and measured mRNA expression and activity of TNF-α. Following exposure for 90 min, an increase in TNF-α mRNA expression was observed. The results, from highest to lowest, were: chrysotile B > chrysotile A > crocidolite > RCF1 > silicon carbide > MMVF21. No increase in expression was seen after exposure to MMVF22. Upregulation of TNF-α activity was seen in response to the two types of chrysotile, MMVF21 and crocidolite, but not with MMVF22, RCF1 or silicon carbide.

Fisher *et al*. (2000) exposed rat alveolar macrophages and monocytes from human peripheral blood to a variety of mineral fibres (3×10^6 fibres/mL) for 16 h and measured

the production of TNF-α protein. Rat macrophages showed increased concentrations of TNF-α protein following exposure to silicon carbide, MMVF10 and RCF1. In human monocytes, increased concentrations of TNF-α protein were observed following exposure to silicon carbide or RCF1.

Cullen *et al*. (1997) added equal numbers of mineral fibres (8.2×10^6/well) to rat alveolar macrophages and measured the increase in production of TNF-α protein. The order of activity was: silicon carbide > crocidolite > long amosite > code 100/475 > MMVF22 > RCF1 and RCF2 > MMVF21 > RCF3 and RCF4.

Gilmour *et al*. (1997) exposed rat alveolar macrophages to amosite asbestos, RCF1 and MMVF10 each at a concentration of 8.24×10^6 fibres/mL and measured the activation of NF-κB and activator protein-1. Nuclear binding of activator protein-1 transcription factor was upregulated by 37%, 9% and 12%, by amosite asbestos, RCF1 and MMVF10, respectively. Only amosite increased the activity of NF-kB.

(*b*) *Mesothelial cells*

Janssen *et al*. (1994) exposed rat pleural mesothelial cells to various concentrations (up to 25 μg/cm^2 area of culture dish) of crocidolite, MMVF10 and RCF1, and determined the steady-state levels of *c-fos* and *c-jun* proto-oncogene mRNA. Changes in *c-fos* and *c-jun* expression induced by crocidolite were seen in response to exposure to concentrations as low as 2.5 μg/cm^2. Exposure to MMVF10 or RCF1 increased expression only at concentrations of 25 μg/cm^2. The potency of MMVF10 and RCF1 was less on a per mass basis than that of crocidolite in this assay.

4.5 Genetic and related effects

4.5.1 *Continuous glass filament and glass wool*

No data were available to the Working Group on the genetic effects of continuous glass filaments.

Asbestos and glass fibres are not direct mutagens in assays with *Escherichia coli* (strain WP2*uvrA*) or *Salmonella typhimurium* (strain TA1538) (Chamberlain & Tarmy, 1977; Barrett *et al*., 1989). However, asbestos fibres have been shown to generate reactive oxygen species and cause DNA damage (strand breaks) in cell-free systems (Gilmour *et al*., 1995; Donaldson *et al*., 1996). In a Syrian hamster embryo cell culture system, exposure to asbestos fibres resulted in accumulation of fibres in the perinuclear region of the cell. Although no gene mutations were detected at two specific loci in these cells (Oshimura *et al*., 1984), it has been proposed that the physical presence of such fibres interferes with chromosome segregation during mitosis, which could result in aneuploidy or chromosomal translocations leading to the activation of proto-oncogenes and/or the inhibition of suppressor genes. The proliferation of such transformed cells could perpetuate genetic errors and lead to oncogenesis (Barrett *et al*., 1989).

Studies on glass fibres have demonstrated a positive response in genotoxicity tests in non-human mammalian cells, as shown by the induction of DNA damage, micronuclei and binucleated and multinucleated cells following exposure to fibres of different origin (Table 73 and references therein). Negative results were obtained with very long and thick fibres; for instance, no genotoxicity was observed in V79 hamster fibroblasts treated with general-purpose building insulation fibres (median length and diameter, 98 μm and 7.3 μm, respectively), whereas treatment with fibres of AAA-10 (median length and diameter, 2 μm and 0.18 μm, respectively) and JM 100 (median length and diameter, 3.5 μm and 0.2 μm, respectively) did produce DNA and chromosomal damage in the same assay. The dependence of micronucleus formation and chromosomal damage on fibre dimensions has been emphasized by numerous independent studies.

Nuclear abnormalities (micronucleus, polynuclei) were investigated in Chinese hamster ovary cells exposed to 17 different samples of glass fibres with average dimensions ranging between 3.5 and 27 μm in length and 0.3 and 7.0 μm in diameter. The effects were directly related to fibre length, whereas diameter had little impact on the induction of nuclear abnormalities. The induction of abnormal anaphases or telophases in rat pleural mesothelial cells was correlated with the presence of fibres — described as the most carcinogenic by Stanton *et al*. (1977) (length, > 8 μm; diameter, ≤ 0.25 μm) — with a non-observable effect level estimated at 2.5×10^5 fibres/cm^2 area of culture dish. Fibres of these dimensions were poorly represented in the MMVF samples used in this assay, possibly accounting for the absence of any activity of MMVF10 or MMVF11.

Formation of micronuclei was observed in Syrian hamster embryo cells exposed to 1 μg/cm^2 of code 100 fibres; cell transformation was also seen under these conditions. Reduction of fibre length by milling, without affecting fibre diameter, diminished the transforming potency of these fibres. Morphological transformation was also found in BALBc-3T3 cells exposed to code 100 fibres.

Formation of binucleated and multinucleated cells was detected after treatment of either untransformed or SV40-immortalized Met5A human mesothelial cells with thin glass wool (median length and diameter, 6.1 μm and 0.29 μm, respectively). Moreover, DNA damage and structural and numerical chromosomal aberrations were observed in human mesothelial cells exposed to glass fibres [not specified].

Many studies have focused on the production of reactive oxygen species by rodent alveolar macrophages or human polymorphonuclear leukocytes treated with glass wool (Table 74 and references). These effects are not necessarily associated with genotoxicity; however, they are cited here because reactive oxygen species may damage DNA in other target cells of the lung (Kane *et al*., 1996). All studies reported production of reactive oxygen species after exposure to glass fibres. In-vitro cell-free assays have been used to investigate the intrinsic ability of fibres to produce reactive oxygen species. Two types of end-point have been used: measurement of guanine hydroxylation in DNA or in deoxyguanosine; or scission of plasmid DNA, as assessed by agarose gel electrophoresis. These assays indicate the formation of hydroxyl radical (• OH) and DNA

Table 73. Genetic and related effects of glass wool

Test system	Result	Dose[a]	Fibre type[b]	Reference
DNA damage (comet assay), Chinese hamster V79 cells	+	1.7 μg/cm^2	Owens Corning AAA-10	Zhong *et al*. (1997)
Formation of micronuclei, Syrian hamster embryo cells	+	1 μg/cm^2 (single dose)	Code 100 (unmilled)	Hesterberg *et al*. (1986
Formation of bi- and multinucleated cells, rat liver epithelial cells	+	1 μg/cm^2 (no dose–response; higher doses had no effect	Thin glass wool	Pelin *et al*. (1995)
Formation of bi- and multinucleated cells, Met-5A and PL102 human mesothelial cells	+	1 μg/cm^2 (higher doses also active)	Thin glass wool	Pelin *et al.* (1995)
Formation of micronuclei and multinucleated cells, Chinese hamster V79 cells	+	10 μg/mL	AAA-10 (Owens Corning); code JM 100	Ong *et al*. (1997)
Formation of micronuclei and multinucleated cells, Chinese hamster V79 cells	–	160 μg/mL	General-purpose building insulation (Owens Corning)	Ong *et al*. (1997)
Nuclear abnormalities (micronucleus and ploidy), Chinese hamster ovary cells	+	Not applicable: data not provided individually	MMVF10; MMVF11; MvL 475/code 90; MvL 475/code 108; MvL 475/code 110; MvL 475/code 112; MvL 475/475TK; MvL 901/FG3; MvL 901/FG5; MvL 901/FG9; MvL 901/FG15; MvL 901/FG19; MvL 901/FG22; MvL 901/FG25; MvL 901/FG31; X607 thick; X607 thin	Hart *et al*. (1994)

Table 73 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
Chromosomal aberrations (structural), Chinese hamster lung cells	–	300 μg/mL	Code 100; code 104; code 108A	Koshi *et al*. (1991)
Chromosomal aberrations (numerical; polyploidy), Chinese hamster lung cells	+	10 μg/mL	Code 100; code 104	Koshi *et al*. (1991)
Chromosomal aberrations (numerical; polyploidy), Chinese hamster lung cells	+	100 μg/mL	Code 108A	Koshi *et al*. (1991)
Cell transformation, Syrian hamster embryo cells	+	2 μg/cm^2	Code 110 (thick)	Hesterberg & Barrett (1984)
Cell transformation, Syrian hamster embryo cells	+[c]	0.5 μg/cm^2	Code 100 (thin)	Hesterberg & Barrett (1984)
Cell transformation, Syrian hamster embryo cells	+[c]	1 μg/cm^2 (single dose)	Code 100 (unmilled)	Hesterberg *et al*. (1986)
Micronucleus formation, Syrian hamster embryo cells	+[c]	1 μg/cm^2 (single dose)	Code 100 (unmilled)	Hesterberg *et al*. (1986)
Cell transformation (foci), anchorage-independent growth of transfectants and transforming potency of transfectants on NIH-3T3, BALB/c-3T3 cells	+	1 μg/cm^2	AAA-10 (Owens Corning)	Gao *et al*. (1995)
Cell transformation (foci), anchorage-independent growth of transfectants and transforming potency of transfectants on NIH-3T3, BALB/c-3T3 cells	+	38 μg/cm^2	General building insulation (Owens Corning)	Gao *et al*. (1995)
Cell transformation (foci), anchorage-independent growth of transfectants and transforming potency of transfectants on NIH-3T3, BALB/c-3T3 cells	+	10 μg/cm^2	Code 100	Gao *et al*. (1995)
8-OH-dG formation, mouse reticulum-cell sarcoma cell line (J774)	–	27 μg/cm^2	Glass	Murata-Kamiya *et al*. (1997)
Anaphase/telophase abnormalities in rat pleural mesothelial cells	–	< 2.5 × 10^5 Stanton fibres/cm$^{2\ d}$	MMVF10; MVVF11	Yegles *et al*. (1995)

Table 73 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
DNA damage (comet assay), human HeI 299 lung fibroblasts	+	3.4 μg/cm^2	Owens Corning AAA-10 microfibres	Zhong *et al*. (1997)
Induction of DNA breakage, repair and interstrand cross-linking, human lung epithelial (A549) cell line	+	40 μg/cm^2 (single dose)	MG1 micro glass fibres (JFMRA); GW1 glass wool fibres (JFMRA)	Wang *et al*. (1999b)
Formation of bi- and multinucleated cells, human MeT-5A mesothelial cells and human primary mesothelial cells from pleural effusions	+	1 μg/cm^2	Thin glass wool	Pelin *et al*. (1995)
Transfection of plasmid and DNA replication, human MeT-5A mesothelial cells	–	1.33 μg/cm^2 (single dose)	Glass fibres	Gan *et al*. (1993)
Chromosomal aberrations in human embryo lung cells	+	1.0 μg/cm^2	MG1 micro glass fibres (JFMRA); GW1 glass wool fibres (JFMRA)	Wang *et al*. (1999b)
Gene amplification and mutation, glass fibre-induced transformed BALB/c-3T3 cells (H-*ras*, K-*ras*, c-*myc* and c-*fos*) (*p53* and *K-ras*)			AAA-10 (Owens Corning)	Whong *et al*. (1999)

8-OH-dG, 8-hydroxydeoxyguanosine

[a] Lowest effective dose or highest ineffective dose

[b] According to the authors

[c] Milling of the fibres strongly reduced the effect.

[d] Defined according to Stanton's criteria: length > 8 μm, diameter ≤ 0.25 μm (Stanton *et al.*, 1977)

Table 74. Other effects of glass wool on cells *in vivo*

Test system	Result	Dose[a]	Fibre type[b]	Reference
Superoxide production in rat alveolar macrophages (fibres either uncoated or coated with rat IgG)	+	3 million fibres (single dose)	Code 100/475	Donaldson *et al*. (1995b)
Superoxide production in rat alveolar macrophages (fibres either uncoated or coated with rat IgG)	+	25 μg[c]	Code 100/475	Hill *et al*. (1996)
Production of reactive oxygen species, superoxide anions, hydrogen peroxide, and reduction of cellular glutathione content in guinea-pig alveolar macrophages	+[d]	200 μg/mL (single dose)	MG1 micro glass fibres (JFMRA); GW1 glass wool fibres (JFMRA)	Wang *et al*. (1999a)
Production of reactive oxygen species, human polymorphonuclear leukocytes	+	200 μg/mL	MMVF11	Luoto *et al*. (1997)
Production of reactive oxygen species, human polymorphonuclear leukocytes	+	100 μg/mL	MMVF10	Luoto *et al*. (1997)
Release of superoxide anions (cytochrome C reduction), hamster alveolar macrophages (+ zymosan)	+	2.5 μg/mL	Code 100	Hansen & Mossman (1987)
Release of superoxide anions (cytochrome C reduction), rat alveolar macrophages	+	5 $\mu g/cm^2$	Code 100	Hansen & Mossman (1987); Mossman & Sesko (1990)
Production of hydrogen peroxide, human polymorphonuclear leukocytes	+	200 μg/mL (single dose)	Glass wool	Leanderson & Tagesson (1992)
8-OH-dG formation (from dG added in the mixture), human polymorphonuclear leukocytes	+	500 μg/mL	Glass wool	Leanderson & Tagesson (1992)
Production of reactive oxygen species (chemiluminescence), human monocytes	+	5×10^5 fibres[c]	GW1 glass wool fibres (JFMRA)	Ohyama *et al*. (2000)

Table 74 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
Production of reactive oxygen species (chemiluminescence), human monocytes	+	35×10^5 fibres[c]	MG1 micro glass fibres (JFMRA)	Ohyama *et al*. (2000)
Production of reactive oxygen species (chemiluminescence), human polymorphonuclear leukocytes	+	500 μg/mL	Glass wool 2; glass wool 3	Ruotsalainen *et al*. (1999)

JFMRA, Japan Fibrous Material Research Association; 8-OH-dG, 8-hydroxydeoxyguanosine; dG, deoxyguanosine
[a] Lowest effective dose or highest ineffective dose
[b] According to the authors
[c] Dose estimated from paper
[d] GW1 fibres did not significantly reduce glutathione content.

breakage, respectively. The results obtained with glass wool show formation of 8-hydroxydeoxyguanosine. The data obtained with plasmids are more difficult to interpret since both positive and negative results have been reported (Table 75).

Hesterberg and Barrett (1984) studied the ability of code 100 (thin) and code 110 (thick) glass fibres to induce cell transformation in Syrian hamster embryo cells. When compared on a per weight basis, thick glass fibres (average diameter, 0.8 ± 0.06 μm) were 20 times less potent than thin fibres (average diameter, 0.13 ± 0.005 μm) in inducing cell transformation. Fibre length was also found to be critical, since milling glass fibres to lengths < 1 μm resulted in a more than 10-fold decrease in the transforming potency. Examination by microscopy indicated that fibres entered the cells and accumulated in the perinuclear regions (Barrett *et al*., 1989). These glass fibres altered chromosomal segregation by blocking cytokinesis (Jensen & Watson, 1999) and gave rise to binucleation or polynucleation in Syrian hamster embryo cells, human mesothelial cells, monkey epithelial cells, Chinese hamster ovary cells and V79 Chinese hamster lung fibroblasts (Oshimura *et al*., 1984; Hart *et al*., 1994; Ong *et al*., 1997; Jensen & Watson, 1999). Binucleation was reported to be directly dependent on the length of the glass fibres and inversely dependent on fibre diameter (Hart *et al*., 1994). Gao *et al*. (1995) demonstrated that transformation of BALB/c-3T3 cells with code 100 or AAA-10 glass fibres resulted in gene transformation, since DNA isolated from fibre-transformed cells showed transforming potency upon transfection into naive cells. It was also demonstrated that thin glass fibres, but not thick fibres, induced cell transformation.

Gao *et al*. (1997) reported that BALB/c-3T3 cells morphologically transformed by AAA-10 glass microfibres overexpressed c-*jun*. Similarly, BALB/c-3T3 cells transformed by AAA-10 glass microfibres showed amplification of the genes H-*ras*, K-*ras*, c-*myc* and c-*fos* in 100, 56, 56 and 67% of the cells examined, respectively (Whong *et al*., 1999). Mutations in the *p53* gene were also noted. These results suggest that activation of proto-oncogenes and inactivation of the *p53* suppressor gene may play a mechanistic role in cell transformation induced by glass fibres.

4.5.2 *Rock (stone) wool and slag wool*

Few data are available on the genotoxic effects of rock (stone) wool and slag wool (Table 76). Increased numbers of revertants were found in *Salmonella typhimurium* TA100 exposed to MMVF21 and the number of revertants was greater in the glutathione-deficient strains TA100/NG-54 and TA100/NG-57. DNA adducts were detected in strain TA104.

Both rock (stone) wool (MMVF21) and slag wool (MMVF22) produced chromosomal abnormalities in a Chinese hamster ovary cell line. Induction of DNA damage (breakage, cross-links) was observed in a human lung cancer cell line, A549 (one dose tested), and chromosomal aberrations were found in human embryonic lung cells.

Increased production of reactive oxygen species has been noted in most of the studies carried out with slag wool and rock (stone) wool and rodent alveolar macro-

Table 75. Other effects of glass wool *in vitro*

Test system	Result	Dose[a]	Fibre type[b]	Reference
Guanine hydroxylation in DNA (potentiation by hydrogen peroxide)	+	10 mg/mL (single dose)	Glass wool	Leanderson & Tagesson (1989)
Guanine hydroxylation in DNA (negative without hydrogen peroxide; potentiation by $FeSO_4$, EDTA and mannitol)	+	2.5 mg/mL	Glass wool	Adachi *et al*. (1992)
Guanine hydroxylation, calf thymus DNA and in dG solution (potentiation by $FeCl_2$ and hydrogen peroxide)	+	10 mg/mL (single dose)	Glass wool	Leanderson *et al*. (1989)
Plasmid φX174, scission of supercoiled DNA	–	46.25×10^6 fibres/mL (single dose)	Code 100/475	Brown *et al*. (1998b)
Plasmid φX174, scission of supercoiled DNA	–	30.8×10^6 WHO fibres/mL (single dose)	MMVF10; MMVF11	Donaldson *et al*. (1995c)
Plasmid φX174, scission of supercoiled DNA	–	61.7×10^6 WHO fibres/mL	MMVF10; MMVF11	Gilmour *et al*. (1995)
Plasmid φX174, scission of supercoiled DNA	+	46.5×10^6 fibres/mL	MMVF10	Gilmour *et al*. (1997)
Plasmid φX174, scission of supercoiled DNA	–	46.25×10^6 fibres/mL (single dose)	MMVF10	Brown *et al*. (1998b)
Hydroxylation of deoxyguanosine	+	NR	Not specified	Leanderson *et al*. (1988)
Hydroxylation of guanine in DNA *in vitro*	+	20 mg (single dose)	Not specified	Leanderson *et al*. (1988)
Hydroxyl radical release in salicylate solution in the presence of hydrogen peroxide	+[c]	1 mg/mL (single dose)	GW Owens Corning; Code 100	Maples & Johnson (1992)
Hydroxyl radical release in salicylate solution	–	8.24×10^7 fibres/mL	Code 100/475	Brown *et al*. (1998b)
Glutathione depletion from rat lung lining fluid	+	8.24×10^6 fibres/mL	MMVF10	Brown *et al*. (2000b)

Table 75 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
Glutathione depletion from rat lung lining fluid	+	4.12×10^7 fibres/mL	Code 100/475	Brown *et al*. (2000b)
Glutathione depletion from pure solution	+	4.12×10^7 fibres/mL	Code 100/475; MMVF10	Brown *et al*. (2000b)
Ascorbate depletion from rat lung lining fluid	+	4.12×10^7 fibres/mL	Code 100/475; MMVF10	Brown *et al*. (2000b)
Ascorbate depletion from pure solution	+	4.12×10^7 fibres/mL	Code 100/475; MMVF10	Brown *et al*. (2000b)

NR, not reported
[a] Lowest effective dose or highest ineffective dose
[b] According to the authors
[c] No effect without hydrogen peroxide

Table 76. Genetic and related effects of slag wool and rock (stone) wool

Test system	Result	Dose[a]	Fibre type[b]	Reference
Salmonella typhimurium TA100, reverse mutation, without exogenous metabolic system	(+)[c]	0.1–0.25 mg/plate	MMVF21	Howden & Faux (1996a)
Formation of fluorescent DNA adducts in *Salmonella typhimurium* TA104	+	40 µg/mL	MMVF21	Howden & Faux (1996b)
Nuclear abnormalities (micronucleus and ploidy), Chinese hamster ovary cells	+	NR	MMVF21; MMVF22	Hart *et al.* (1994)
Induction of DNA breakage, inhibition of DNA repair and DNA interstrand cross-linking, human lung epithelial (A549) cells	+	40 µg/cm^2 (single dose)	RW1 rock (stone) wool (slag wool, RW1; JFMRA)	Wang *et al.* (1999b)
Chromosomal aberrations, human embryo lung cells	+	1.0 µg/cm^2	RW1 rock (stone) wool (slag wool, RW1; JFMRA)	Wang *et al.* (1999b)

NR, not reported
[a] Lowest effective dose or highest ineffective dose
[b] According to the authors
[c] Higher number of revertants in glutathione-deficient strains

phages or human polymorphonuclear leukocytes (Table 77). Moreover, lipid peroxidation was detected in *S. typhimurium* TA104 and rat lung fibroblasts exposed to MMVF21. Glass wool, slag wool and rock (stone) wool all induced guanosine hydroxylation and a low level of DNA breakage (Table 78). Inconclusive results were obtained in DNA plasmid scission tests.

4.5.3 *Refractory ceramic fibres*

Genetic effects of refractory ceramic fibres have been reported (see Table 79 for details and references). Fluorescent malondialdehyde–DNA adducts were observed in *S. typhimurium* TA104 exposed to RCF-1. Aneuploidy was observed in *Drosophila melanogaster* fed with different samples of refractory ceramic fibres. However, no dose–response relationships were reported in these assays. In studies in non-human mammalian cells, nuclear abnormalities were observed after exposure of Chinese hamster ovary cells to RCF1, RCF2, RCF3 and RCF4. As with glass wool, the effect on nuclear abnormalities (micronuclei and polynuclei) was directly related to fibre length. In this assay, the average length of the refractory ceramic fibres ranged between 9.2 μm and 24.3 μm and the average diameter from 1.0 μm–1.4 μm.

Negative results were obtained in studies in which other non-human mammalian cells were exposed to refractory ceramic fibres. No abnormalities of anaphase or telophase were observed in rat pleural mesothelial cells treated with different samples of refractory ceramic fibres. As mentioned for glass wool, this absence of genotoxic effects may be related to the small number of Stanton fibres (length > 8 μm; diameter ≤ 0.25 μm) present in these samples. No deoxyguanosine hydroxylation was detected in a reticulum-cell sarcoma cell line (J774) exposed to a sample of refractory ceramic fibres and no mutagenicity was detected at the *Hprt* or *S1* locus in human–hamster hybrid A_L cells following exposure to RCF1. In this experiment, the total numbers of fibres at the highest ineffective dose (20 or 40 μg/cm^2) were 2×10^5 fibres and 4×10^5 fibres [according to the data provided in the paper]. As the percentage of Stanton fibres is probably small, the cells in this study may have been exposed to an insufficient number of effective fibres.

The refractory ceramic fibres RCF1, RCF2 and RCF3 caused DNA damage (breakage and cross-links) in a human lung tumour epithelial cell line A549 (one dose investigated). Micronucleus formation and structural and numerical chromosomal aberrations were detected in human amniotic fluid cells and structural chromosomal aberrations were noted in human embryonic lung cells.

All studies conducted on rodent alveolar macrophages and human polymorphonuclear leukocytes exposed to refractory ceramic fibres demonstrated the production of reactive oxygen species (Table 80). Results of the hydroxyl radical generation assay were reported in two studies in which only one dose was tested (Table 81). The results obtained with the DNA plasmid scission assay are inconclusive since both negative and weakly positive effects have been reported.

Table 77. Other effects of slag wool and rock (stone) wool in cultured cells

Test system	Result	Dose[a]	Fibre type[b]	Reference
Lipid peroxidation, formation of malondialdehyde-DNA adducts, *Salmonella typhimurium* TA104	+	40 µg/mL	MMVF21	Howden & Faux (1996b)
Production of reactive oxygen species, superoxide anion production, hydrogen peroxide production in guinea-pig alveolar macrophages	+	200 µg/mL (single dose)	RW1 rock (stone) wool (slag wool, JFMRA)	Wang *et al*. (1999a)
Lipid peroxidation, rat lung RFL-6 fibroblasts	+	2 µg/cm^2	MMVF21	Howden & Faux (1996b)
Superoxide anion production, rat alveolar macrophages (fibres either coated or not coated with rat IgG)	+[c]	3 million fibres (single dose)	MMVF21	Donaldson *et al*. (1995b)
Superoxide anion production, rat alveolar macrophages (fibres either coated or not coated with rat IgG)	+[c]	12.5 µg	MMVF21	Hill *et al*. (1996)
Haemolysis, sheep erythrocytes	+	2.5 mg/mL	MMVF21; MMVF22	Luoto *et al*.(1997)
Viability of rat alveolar macrophages in suspension (LDH release)	+	1 mg/mL (single dose)	MMVF21; MMVF22	Luoto *et al*. (1997)
Production of reactive oxygen species, human poly-morphonuclear leukocytes	+	100 µg/ml	MMVF21; MMVF22	Luoto *et al*. (1997)
Hydrogen peroxide production by human polymorphonuclear leukocytes	+	200 µg/mL (single dose)	Rockwool I; rockwool II	Leanderson & Tagesson (1992)
8-OH-dG formation (from dG added in the mixture) from polymorphonuclear leukocytes	+	500 µg/mL	Rockwool I; rockwool II	Leanderson & Tagesson (1992)
Production of reactive oxygen species (chemiluminescence) by human monocyte-derived macrophages	+	5×10^5 fibres	RW1 rock (stone) wool (JFMRA)	Ohyama *et al*. (2000)

Table 77 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
Production of reactive oxygen species (chemiluminescence) by human polymorphonuclear leukocytes	+	500 μg/mL	Rockwool 4; Rockwool 5; Rockwool 6	Ruotsalainen *et al.* (1999)
Manganese superoxide dismutase (MnSOD) mRNA induction, MnSOD enzyme activity induction, SV40-transformed human bronchial epithelial (BEAS 2B) cells	+	2 μg/cm^2 [d]	Rockwool 115-4	Marks-Konczalik *et al*. (1998)

LDH, lactate dehydrogenase; 8-OH-dG, 8-hydroxydeoxyguanosine; dG, deoxyguanosine

[a] Lowest effective dose or highest ineffective dose

[b] According to the authors

[c] Coated fibres were much more active than uncoated fibres.

[d] A decrease is observed at fibre concentrations ≥ 25 μg/cm^2.

Table 78. Other effects of slag wool and rock (stone) wool *in vitro*

Test system	Result	Dose[a]	Fibre type[b]	Reference
Guanine hydroxylation	+	NR	Rock (stone) wool	Leanderson *et al*. (1988)
Guanine hydroxylation in calf thymus DNA	+	10 mg/mL (single dose)	Rock (stone) wool	Leanderson *et al*. (1988)
Guanine hydroxylation (potentiation by hydrogen peroxide and $FeCl_2$), calf thymus DNA and in dG solution	+	10 mg/mL (single dose)	Rock (stone) wool	Leanderson *et al*. (1989)
Guanine hydroxylation in calf thymus DNA and in dG solution	+	10 mg/mL (single dose)	Slag wool	Leanderson *et al*. (1989)
Plasmid φX174, scission of supercoiled DNA	–	30.8×10^6 WHO fibres/mL (single dose)	MMVF21; MMVF22	Donaldson *et al*. (1995c)
Plasmid φX174, scission of supercoiled DNA	–	61.7×10^6 WHO fibres/mL	MMVF21; MMVF22	Gilmour *et al*. (1995)
Plasmid φX174, scission of supercoiled DNA	(+)	NR	MMVF21; MMVF22	Donaldson *et al*. (1996)
Formation of 8-OH-dG, calf thymus DNA	+	0.5 mg/mL	MMVF21	Howden & Faux (1996a)
Oxidative potential (ethylene formation from α-keto-γ-methiol butyric acid)	+	4 mg/mL (single dose)	MMVF13	Hippeli *et al*. (1997)

dG, deoxyguanosine; 8-OH-dG, 8-hydroxydeoxyguanosine; NR, not reported
[a] Lowest effective dose or highest ineffective dose
[b] According to the authors

Table 79. Genetic and related effects of refractory ceramic fibres

Test system	Result	Dose[a]	Fibre type[b]	Reference
Formation of malondialdehyde-DNA adducts (lipid peroxidation), *Salmonella typhimurium* TA104	+	40 μg/mL (single dose)	RCF1	Howden & Faux (1996b)
Aneuploidy, *Drosophila melanogaster*, adult females	+	25 mg/mL in feed (single dose)	RCF1 (MTC); RCF2 (MTC); RCF3 (MTC); RCF4 (MTC)	Osgood (1994)
Aneuploidy, *Drosophila melanogaster*, larvae	+	250 mg/bottle (single dose)	RCF1 (MTC); RCF3 (MTC)	Osgood (1994)
Aneuploidy, *Drosophila melanogaster*, larvae	–	250 mg/bottle (single dose)	RCF2 (MTC); RCF4 (MTC)	Osgood (1994)
8-OH-dG formation, mouse reticulum sarcoma (J774) cell line	–	27 $\mu g/cm^2$	Refractory ceramic fibres	Murata-Kamiya *et al*. (1997)
Formation of malondialdehyde-DNA adducts (lipid peroxidation), rat lung (RFL-6) fibroblasts	–	5 $\mu g/cm^2$	RCF1	Howden & Faux (1996b)
Gene mutation, human–hamster hybrid A_L cells, *HPRT* locus	–	40 $\mu g/cm^2$	RCF1 (TIMA)	Okayasu *et al*. (1999)
Gene mutation, human–hamster hybrid A_L cells, *S1* locus	–	20 $\mu g/cm^2$	RCF1 (TIMA)	Okayasu *et al*. (1999)
Micronucleus formation and apoptosis, Syrian hamster embryo cells	+	10 $\mu g/cm^2$	Refractory ceramic fibres	Dopp *et al.* (1995)
Nuclear abnormalities (micronucleus and polynucleus formation), Chinese hamster ovary (K1) cells	+	5 $\mu g/cm^2$	RCF1; RCF2; RCF3; RCF4	Hart *et al*. (1992, 1994)
Anaphase/telophase abnormalities in rat pleural mesothelial cells	–	$> 2.5 \times 10^5$ Stanton fibres/cm^2 (threshold dose)[c]	RCF1; RCF3; RCF4 (TIMA)	Yegles *et al*. (1995)

Table 79 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
Micronucleus formation, human amniotic fluid cells or Syrian hamster embryo cells	+	0.5 μg/cm^2	Refractory ceramic fibres	Dopp *et al*. (1997); Dopp & Schiffmann (1998)
Chromosome breakage and hyperdiploidy, human amniotic fluid cells	+	5.0 μg/cm^2 (single dose)	Refractory ceramic fibres	Dopp *et al*. (1997)
Induction of DNA breakage, DNA repair and DNA interstrand cross-linking, human lung epithelial tumour (A549) cell line	+	40 μg/cm^2 (single dose)	RF1, RF2, RF3 (JFMRA)	Wang *et al*. (1999b)
Chromosomal aberrations, human embryo lung cells	+	1.0 μg/cm^2	RF1, RF2, RF3 (JFMRA)	Wang *et al*. (1999b)

8-OH-dG, 8-hydroxydeoxyguanosine

[a] Lowest effective dose or highest ineffective dose

[b] According to the authors; MTC, Mountain Technical Center, Littleton, CO; TIMA, Thermal Insulation Manufacturers' Association

[c] Defined according to Stanton's criteria: length > 8 μm, diameter ≤ 0.25 μm (Stanton *et al.*, 1977)

Table 80. Other effects of refractory ceramic fibres in cultured cells

Test system	Result	Dose[a]	Fibre type[b]	Reference
Superoxide production, rat alveolar macrophages (fibres either uncoated or coated with rat IgG)	+	3×10^6 (single dose)	RCF1	Donaldson *et al*. (1995b)
Superoxide production, rat alveolar macrophages (fibres either uncoated or coated with rat IgG)	+	125 µg	RCF1	Hill *et al*. (1996)
Production of reactive oxygen species, human polymorphonuclear leukocytes	+	100 µg/mL	RCF1, RCF2, RCF3	Luoto *et al.* (1997)
Production of reactive oxygen species in human polymorphonuclear leukocytes	+	200 µg/mL	RCF4	Luoto *et al*. (1997)
Production of reactive oxygen species, superoxide anion production, guinea-pig alveolar macrophages	+	200 µg/mL (single dose)	RF1, RF2, RF3 (JFMRA)	Wang *et al*. (1999a)
Reduction of cellular glutathione content, guinea-pig alveolar macrophages	+	200 µg/mL (single dose)	RF2 (JFMRA)	Wang *et al.* (1999a)
Reduction of cellular glutathione content, guinea-pig alveolar macrophages	–	200 µg/mL (single dose)	RF1, RF3 (JFMRA)	Wang *et al.* (1999a)
Reduction of cellular glutathione content, rat alveolar macrophages	+	8.24×10^6 fibres/mL (single dose)	RCF1	Gilmour *et al*. (1997)
Regulation of trancription factors (gel mobility shift assay) for activator protein-1, rat alveolar macrophages	+	8.24×10^6 fibres/mL (single dose)	RCF1	Gilmour *et al*. (1997)
Regulation of trancription factors (gel mobility shift assay) for NFκB, rat alveolar macrophages	–	8.24×10^6 fibres/mL (single dose)	RCF1	Gilmour *et al*. (1997)
Hydrogen peroxide production, human polymorphonuclear leukocytes	+	200 µg/mL (single dose)	Refractory ceramic fibre (Kerlane®)	Leanderson & Tagesson (1992)
8-OH-dG formation (from dG added in the mixture), human polymorphonuclear leukocytes	+	500 µg/mL	Refractory ceramic fibre (Kerlane®)	Leanderson & Tagesson (1992)

Table 80 (contd)

Test system	Result	Dose[a]	Fibre type[b]	Reference
Production of reactive oxygen species (chemiluminescence), human polymorphonuclear leukocytes	+	500 µg/mL	Ceramic fibre 1; ceramic fibre 7	Ruotsalainen *et al.* (1999)
Production of reactive oxygen species, human monocyte-derived macrophages	+	10×10^5 fibres	RF1, RF2 (JFMRA)	Ohyama *et al.* (2000)
Production of reactive oxygen species, human monocyte-derived macrophages	+	30×10^5 fibres	RF3 (JFMRA)	Ohyama *et al.* (2000)

8-OH-dG, 8-hydroxydeoxyguanosine; dG, deoxyguanosine
[a] Lowest effective dose or highest ineffective dose
[b] According to the authors

Table 81. Other effects of refractory ceramic fibres *in vitro*

Test system	Result	Dose[a]	Fibre type[b]	Reference
Plasmid φX174, scission of supercoiled DNA	–	30.8×10^6 WHO fibres/mL (single dose)	RCF1, RCF2, RCF3, RCF4	Donaldson *et al*. (1995c)
Plasmid φX174, scission of supercoiled DNA	(+)	61.7×10^6 fibres/mL	RCF1, RCF3, RCF4	Gilmour *et al*. (1995)
Plasmid φX174, scission of supercoiled DNA	–	61.7×10^6 fibres/mL (single dose)	RCF2	Gilmour *et al*. (1995)
Plasmid φX174, scission of supercoiled DNA	(+)	NR	RCF1, RCF2, RCF3, RCF4	Donaldson *et al*. (1996)
Plasmid φX174, scission of supercoiled DNA	+	46.5×10^6 fibres/mL (single dose)	RCF1	Gilmour *et al*. (1997)
Plasmid φX174, scission of supercoiled DNA	–	46.25×10^6 fibres/mL (single dose)	RCF1, RCF4	Brown *et al*. (1998b)
Hydroxyl radical release in salicylate solution	+	8.24×10^7 fibres/mL (single dose)	RCF1	Brown *et al*. (1998b)
Hydroxyl radical release in salicylate solution	–	8.24×10^7 fibres/mL (single dose)	RCF4	Brown *et al*. (1998b)

NR, not reported
[a] Lowest effective dose and highest ineffective dose
[b] According to the authors