# 6 | Discussion and Summary of Fiber Toxicity

## 6.1 Significance of Studies with RCFs

Three major sources of data contributing to the literature on RCFs are (1) experimental studies with animals and in vitro bioassays, (2) epidemiologic studies of populations with occupational exposure to RCFs (primarily during manufacturing), and (3) exposure assessment studies that provide quantitative and qualitative measurements of exposures as well as the physical and chemical characteristics of airborne RCFs. Each of these sources of information is considered integral to this criteria document for providing a more comprehensive evaluation of occupational exposure to RCFs and their potential health consequences.

Data from inhalation studies with animals exposed to RCFs have demonstrated statistically significant increases in the induction of lung tumors in rats and mesotheliomas in hamsters [Mast et al. 1995a,b; McConnell et al. 1995]. Other inhalation studies with RCFs have shown pathobiologic inflammatory responses in lung and pleural tissues [Gelzleichter et al. 1996a,b]. Implantation and instillation methods have also been used in animal studies with RCFs to determine the potential effects of these fibers on target tissues. These studies have recognized limitations for interpreting results because the exposure techniques bypass the natural defense and clearance mechanisms associated with the normal route of exposure (i.e., inhalation). However, they are useful for demonstrating mechanisms of toxicity and comparative measures of toxicity for different agents. RCFs implanted into the pleural and abdominal cavities of various strains of rats and hamsters have produced mesotheliomas, sarcomas, and carcinomas at the sites of fiber implantation [Wagner et al. 1973; Davis et al. 1984; Pott et al. 1987]. Similar tumorigenic responses have been observed following intratracheal instillation of RCFs [Manville Corporation 1991]. These data provide additional evidence of the carcinogenic effects of RCFs in exposed laboratory animals.

Epidemiological data have not associated occupational exposure to RCFs under current exposure conditions with increased incidence of pleural mesothelioma or lung cancer [Lockey et al. 1993; Lemasters et al. 1998]. However, in epidemiologic studies of workers in RCF manufacturing facilities [Lemasters et al. 1994; Lockey et al. 1993, 1996; Rossiter et al. 1994; Trethowan et al. 1995; Burge et al. 1995; Cowie et al. 1999], increased exposures to airborne fibers have been linked to pleural plaques, small radiographic parenchymal opacities, decreased pulmonary function, respiratory symptoms and conditions (pleurisy, dyspnea, cough), and skin and eye irritation.

Many of the respiratory effects showed a statistically significant association with RCF exposure after controlling or adjusting for potential confounders, including cigarette smoking and exposure to nonfibrous dust. Yet in PFTs, the interactive effect between smoking and RCF exposure was especially pronounced, based on the finding that RCF-associated decreases

in pulmonary function were limited to current and former smokers [Lockey et al. 1998; Lemasters et al. 1998; Trethowan et al. 1995; Burge et al. 1995]. The interactive effect between exposure to airborne fibers and cigarette smoke has been previously documented (e.g., Selikoff et al. [1968] ). However, unlike male workers, nonsmoking female workers did show statistically significant decreases in PFT results associated with RCF exposure [Lemasters et al. 1998]. Analyses of data from multiple PFT sessions [Lockey et al. 1998] have led researchers to conclude that decreases in pulmonary function were more strongly influenced by the higher exposures to airborne RCFs that occurred in the past. This conclusion seems plausible, since historical air-sampling data indicate that airborne fiber concentrations were much higher in the first decades of RCF manufacturing and that former workers had potentially higher exposures.

Multiple studies have been performed to characterize the concentrations and characteristics of airborne exposures to RCFs in the workplace. Current and historical environmental monitoring data [Esmen et al. 1979; Cantor and Gorman 1987; Gorman 1987; O'Brien et al. 1990; Cheng et al. 1992; Brown 1992; Corn et al. 1992; Lyman 1992; Allshouse 1995; Hewett 1996] indicate that airborne exposures to RCFs include fibers in the respirable size range (<3.5 $\mu$m in diameter and <200 $\mu$m long [Timbrell 1965; Lippmann 1990; Baron 1996]). These exposures occur in primary RCF manufacturing as well as in secondary industries such as RCF installation and removal. Sampling data from studies of domestic, primary RCF manufacturing sites indicate that average airborne fiber concentrations have steadily declined by nearly 2 orders of magnitude over the past 2 decades. For example, Rice et al. [1997] report a maximum exposure estimate of 10 f/cm$^3$ associated with an RCF manufacturing process in the 1950s, and Esmen et al. [1979] measured

average exposure concentrations ranging from 0.05 to 2.6 f/cm$^3$ in RCF facilities in the middle to late 1970s. Rice et al. [1994, 1996, 1997] suggest average concentrations in manufacturing ranging from <LOD to 0.66 f/cm$^3$ in the late 1980s, and Maxim et al. [1994, 1997, 2000a] report that concentrations from the late 1980s through 1997 ranged from an AM of <0.3 to 0.6 f/cm$^3$ (GM≈0.2 f/cm$^3$). For many manufacturing processes, even greater reductions in exposures have been realized through improved ventilation, engineering or process changes, and product stewardship programs [Rice et al. 1996; Maxim et al. 1999b].

Although the potential exists for exposure to respirable crystalline silica in the forms of quartz, tridymite, and cristobalite during work with RCFs, exposure monitoring data indicate that these exposures are generally low [Rice et al. 1994]. Maxim et al. [1999b] report that many airborne samples of crystalline silica collected during the installation and removal of RCF products contain concentrations below the LOD, with average concentrations of respirable crystalline silica per measurable task ranging from 0.01 to 0.44 mg/m$^3$ (equivalent 8-hr TWA range=0.004 to 0.148 mg/m$^3$). Other studies have shown greater potential for exposure to respirable crystalline silica (especially in the form of cristobalite) during the removal of after-service RCF materials [Gantner 1986; Cheng et al. 1992; Perrault et al. 1992; van den Bergen et al. 1994; Sweeney and Gilgrist 1998]. For processes associated with higher concentrations of airborne respirable fibers, there are also generally greater concentrations of total and respirable dusts [Esmen et al. 1979; Krantz et al. 1994].

## 6.2 Factors Affecting Fiber Toxicity

To accurately interpret the results of experimental and epidemiologic studies with RCFs,

it is important to consider recognized factors that contribute to fiber toxicity for RCFs and other SVFs in general. The major determinants of fiber toxicity have been identified as fiber dose (or its surrogate, airborne fiber exposure), fiber dimensions (length and diameter), and fiber durability (especially as it affects fiber biopersistence in the lungs) [Bignon et al. 1994; Bunn et al. 1992; Bender and Hadley 1994; Christensen et al. 1994; Lockey and Wiese 1992; Moore et al. 2001].

## 6.2.1  Fiber Dose

The measurement of airborne fiber concentrations is frequently used as a surrogate for assessing dose and health risk to workers. Analyses of historical and current air sampling data indicate that occupational exposure concentrations of airborne RCFs have decreased dramatically in the manufacturing sector [Maxim et al. 1997; Rice et al. 1997]. In chronic inhalation studies of RCFs [Mast et al. 1995a,b; McConnell et al. 1995], both rats and hamsters were exposed to a range of size-separated RCF concentrations in a nose-only inhalation protocol. When airborne RCFs are generated, half or more of the aerosol is composed of respirable particles of unfiberized material that was formerly a component of the fiber [Mast et al. 1995a,b]. Because of the nature of this mixed exposure, it is difficult to determine the relative contributions of the airborne fibers and nonfibrous particulates to the adverse effects observed in humans and animals. It has been postulated that the nonfibrous particulates may have contributed to an overload effect in the Mast et al. [1995a,b] animal studies with RCFs [Yu et al. 1994; Mast et al. 1995a,b; Maxim et al. 1997; Brown et al. 2000]. Burge et al. [1995] have suggested that the health effects seen in RCF-exposed workers are a consequence of combined particulate and fiber exposure, but the decrements in lung function are more related to fiber exposure combined with smoking.

Other studies have shown that for processes associated with higher concentrations of airborne respirable fibers, there is also a greater concentration of total and respirable dust [Esmen et al. 1979; Krantz et al. 1994].

## 6.2.2  Fiber Dimensions

Throughout the literature, studies support the theory that fiber toxicity is related to fiber dimensions [Timbrell 1982, 1989; Harris and Timbrell 1977; Stanton et al. 1977, 1981; Lippmann 1988]. Initially, fiber dimensions (length and diameter) play a significant role in determining the deposition site of a fiber in the lungs. Longer and thicker (>3.5 μm in diameter) fibers are preferentially deposited in the upper airways by the mechanisms of impaction [Yu et al. 1986] or interception. Timbrell [1965] suggested that direct interception plays an important role in the deposition of fibers, as the fiber comes into contact with the airway wall and is deposited. Fibers being deposited in the larger ciliated airways are generally cleared via the mucociliary escalator. Thinner fibers tend to maneuver past airway bifurcations into smaller and smaller airways until their dimensions dictate deposition either by sedimentation or diffusion [Asgharian and Yu 1989]. Another factor that may enhance deposition is the electrostatic charge a fiber can accumulate during dust-generating processes in occupational settings [Vincent 1985]. The fiber charge may affect its attraction to the lung surface, causing the fiber to be deposited by electrostatic precipitation.

Although the dimensional characteristics and geometry of a fiber influence its deposition in the respiratory tract, the fiber's length and chemical properties dictate its clearance and retention once it has been deposited within the alveolar region. For the fiber that traverses the respiratory airways and is deposited in the gas exchange region, possible fates include

dissolution, clearance via phagocytic cells (alveolar macrophages) in the alveoli, or translocation through membranes into interstitial tissues. Both test animals and workers have been exposed to RCFs of similar length and diameter [Allshouse 1995], and these exposures include fibers of respirable dimensions [Esmen et al. 1979; Lockey et al. 1990; Cheng et al. 1992]. Since rats and other rodents are obligate nasal breathers, fibers greater than about 1 μm in diameter are too large for deposition in their alveoli [Jones 1993]. By comparison, humans can inhale and deposit fibers up to 3.5 μm in diameter in the thoracic and gas exchange regions of the lung. This physiological difference prevents the evaluation of fibers with diameters of about 1 to 3.5 μm (which would have human relevance) in rodent inhalation studies.

The role of fiber size in inducing biological effects is well documented and reviewed in the literature [Stanton et al. 1977, 1981; Pott et al. 1987; Warheit 1994]. Stanton et al. [1977] hypothesized that glass fibers longer than 8 μm with diameters thinner than 0.25 μm had high carcinogenic potential. In a review of the significance of fiber size to mesothelioma etiology, Timbrell [1989] concluded that the thinner fibers with an upper diameter limit of 0.1 μm are more potent for producing diseases of the parietal pleura (e.g., mesothelioma and pleural plaques) than thicker fibers. That value for fiber diameter is cited by Lippmann [1988] in his asbestos exposure indices for mesothelioma. Oberdörster [1994] studied the effects of both long (>10–16 $\mu$m) and short (<10 $\mu$m) fibers on alveolar macrophage functions, concluding that both will lead to inflammatory reactions—although a distinct difference exists in the long-term effects because of differential clearance of fibers of different sizes. Alveolar macrophages constitute the first line of defense against particles deposited in the alveoli; they migrate to sites where fibers are deposited and phagocytize them. The engulfed fibers are then

moved by the macrophages toward the mucociliary escalator and removed from the respiratory tract. The ability of the macrophages to clear fibers is size-dependent. Short fibers (<15 μm long) can usually be phagocytized by one rat alveolar macrophage [Luoto et al. 1994; Morgan et al. 1982; Oberdörster et al. 1988, Oberdörster 1994], whereas longer fibers may be engulfed by two or more macrophages. Blake et al. [1998] have suggested that incomplete or frustrated phagocytosis may play a role in the increased toxicity of longer fibers. Fiber length has been correlated with the cytotoxicity of glass fibers [Blake et al. 1998], with greatest cytotoxicity for fibers 17 and 33 μm long compared with shorter fiber samples. Long fibers (17 $\mu$m average length) tend to be a more potent inducer of TNF production and transcription factor activation than short fibers (7 $\mu$m average length) [Ye et al. 1999].

When comparing the dimensions of airborne fibers with those found in the lungs, it is important to consider the preferential clearance of shorter fibers as well as the effects of fiber dissolution and breakage. Yu et al. [1996] evaluated these factors in a study that led to the development of a clearance model for RCFs in rat lungs. Results of that study confirmed that fibers 10 to 20 μm long are cleared more slowly than those <10 μm long because of the incomplete phagocytosis of long fibers by macrophages. The preferential clearance of shorter fibers has also been documented in studies with chrysotile asbestos and other mineral fibers, in which the average length of retained fibers increased during a discrete period following deposition [Coin et al. 1992; Churg 1994]. This increase might also be explained by the longitudinal cleavage pattern of asbestos fibers, which results in longer fibers of decreasing diameters [Coin et al. 1992]. By contrast, any breakage of RCFs would occur perpendicular to the longitudinal plane, resulting in shorter fibers of the same diameter. For the clearance

model developed by Yu et al. [1996], the effect of fiber breakage was also assessed from experimental data and incorporated into the model. The authors concluded that the simultaneous effect of fiber breakage and differential clearance leads only to a small change in fiber size distribution in the lung. This result suggests that the dimensions of fibers in the lung are closely related to the dimensions of fibers measured in the airborne samples (adjusted for deposition); thus, most short fibers in the lungs originated as short fibers in airborne exposures.

The dimensions of airborne fibers have also been characterized for workers with occupational exposure to RCFs. One study of domestic RCF manufacturing facilities found that approximately 90% of airborne fibers were <3 $\mu$m in diameter, and 95% of airborne fibers were <4 $\mu$m in diameter and <50 $\mu$m long [Esmen et al. 1979]. The study showed that diameter and length distributions of airborne fibers in the facilities were consistent with a $GM_D$ of 0.7 $\mu$m and a $GM_L$ of 13 $\mu$m. Another air sampling study of domestic RCF manufacturing sites reported that 99.7% of the fibers had diameters of <3 $\mu$m and 64% had lengths >10 $\mu$m [Allshouse 1995]. Measurements of airborne fibers in the European RCF manufacturing industry are comparable: Rood [1988] reported that all fibers observed were in the thoracic and respirable size range (i.e., diameter <3 $\mu$m), with median diameters ranging 0.5 to 1.0 $\mu$m and median lengths from 8 to 23 $\mu$m. During removal of RCF products, Cheng et al. [1992] found that 87% of airborne fibers were within the respirable size range, with fiber diameters ranging from 0.5 to 6 $\mu$m (median diameter=1.6 $\mu$m) and fiber lengths ranging from 5 to 220 $\mu$m. Another study [Perrault et al. 1992] of airborne fiber dimensions measured during installation and removal of RCF materials in industrial furnaces reported $GM_D$ values of 0.38 and 0.57 $\mu$m, respectively.

### 6.2.3 Fiber Durability

Biopersistence (and specifically the retention time of the fiber in the lungs) is considered to be an important predictor of fiber toxicity. Fiber solubility affects the biopersistence of fibers deposited within the lung and is a key determinant of fiber toxicity. Bender and Hadley [1994] suggest that some of the important considerations of fiber durability include the following:

- Fiber size—particularly length as it relates to the dimensions of the alveolar macrophages

- Fiber dissolution rate

- Mechanical properties of the fibers, including partially dissolved and/or digested fibers

- Overloading of the normal clearance mechanisms of the lung

Bignon et al. [1994] argue that fibers that are biopersistent in vivo and in vitro are more biologically active than less durable fibers.

The durability of RCFs [Hammad et al. 1988; Luoto et al. 1995] provides a basis for suggesting that these fibers might persist long enough to induce biological effects similar to those of asbestos. In vitro durability tests have shown RCFs to be highly resistant to dissolution in biologically relevant mixtures such as Gamble's solution [Scholze and Conradt 1987]. The persistence of RCFs in both the peritoneal cavity [Bellman et al. 1987] and the lung [Hammad et al. 1988] has been recognized in experimental studies. Hammad et al. [1988] sacrificed rats exposed to either slag wool or ceramic fibers via inhalation at 5, 30, 90, 180, or 270 days after exposure. The lungs of the animals were ashed in a low-temperature asher, and the fiber content of the lungs was evaluated by PCM. The researchers found that 24% of the

deposited RCFs persisted in the lungs of rats sacrificed 270 days following exposure. In the same study, the lungs of rats exposed to slag wool contained only 6% of the slag wool fibers 270 days after exposure compared with those sacrificed 5 days following inhalation. From these results, it was concluded that RCFs follow a clearance pattern of relatively durable fibers that persist, translocate, or are removed by some mechanism other than dissolution. Similar results were obtained in the study by Mast et al. [1995b], which shows that RCFs are persistent in the lungs of rats exposed by inhalation. Specifically, compared with the fiber burden in the lungs of animals sacrificed 3 months after exposure (recovery), the lungs of animals sacrificed after 21 months of recovery contained approximately 20% of the deposited fibers. Of the retained fibers (measured with both SEM and TEM techniques) 54% to 75% had diameters <0.5 μm, and more than 90% were 5 to 20 μm long.

Researchers have suggested that fibers deposited in the gas exchange region with lengths less than the diameter of an alveolar macrophage are phagocytized and cleared via the mucociliary system or the lymph channels. Dissolution of fibers within the AlM occurs if the fibers are not resistant to the acidic intracellular conditions or a pH~5 [Nyberg et al. 1989]. Fibers that are not engulfed by alveolar macrophages are subjected to a pH of 7.4 in the extracellular fluid of the lung. A study of SVF durability in rat alveolar macrophages reports that RCFs are much less soluble than glass wool and rock wool fibers based on the amounts of silicon (Si) and iron (Fe) dissolved from the fibers in vitro [Luoto et al. 1995]. RCFs in rat alveolar macrophage culture dissolved less than 10 mg $Si/m^2$ of fiber surface area and less than 1 mg $Fe/m^2$ of fiber surface area. Glass wool dissolved more than 50 mg $Si/m^2$, and rock wool dissolved nearly 2 mg $Fe/m^2$ when measured over comparable

time periods. However, degradation and dissolution of deposited RCFs may still occur, based on the findings of higher dissolution of aluminum (Al) from RCFs (0.8 to 2.4 mg $Al/m^2$) in alveolar macrophages than from the other SVFs [Luoto et al. 1995]. In another study, SEM analysis of fibers recovered from the lungs of rats 6 months after inhalation of RCFs revealed an eroded appearance, causing the researchers to conclude that dissolution of Si in the fibers is a plausible mechanism for long-term fiber clearance [Yamato et al. 1994].

SVFs in general are less durable than asbestos fibers. RCFs are more durable than many other SVFs, with a dissolution rate somewhat higher than chrysotile asbestos. Under the extracellular conditions in the lung, chrysotile—the most soluble form of asbestos—has a dissolution rate of <1 to 2 $ng/cm^2/hr$. RCFs have a similar dissolution rate of about 1 to 10 $ng/cm^2/hr$ under conditions experienced in pulmonary interstitial fluid. Other more soluble SVFs can be 10 to 1,000 times less durable [Scholze and Conradt 1987; Christensen et al. 1994; Maxim et al. 1999b; Moore et al. 2001]. At the measured solubility rate, an RCF with a 1-μm diameter would take more than 1,000 days to dissolve completely [Leineweber 1984].

## 6.3  Summary of RCF Toxicity and Exposure Data

In addition to the main determinants of fiber toxicity (dose, dimension, and durability), other factors such as elemental composition, surface area, and composition can also influence the toxicity of the fiber. Thus, it is difficult to predict a fiber's potential for human toxicity based solely on in vitro or in vivo tests. Based on consideration of these factors, the major findings from the RCF animal and human studies are as follows:

■ Toxicologic evidence from experimental inhalation studies indicates that RCFs are capable of producing lung tumors in laboratory rats and mesotheliomas in hamsters [Mast et al. 1995a,b; McConnell et al. 1995]. However, interpreting these studies with regard to RCF potency and its implication for occupationally exposed human populations is complicated by the issue of coexposure to fibers and nonfibrous respirable particulate.

■ The durability of RCFs contributes to the biopersistence of these fibers both in vivo and in vitro [Bellmann et al. 1987; Scholze and Conradt 1987; Lockey and Wiese 1992].

■ Cytotoxicity and genotoxicity studies indicate that RCFs

— are capable of inducing enzyme release and cell hemolysis [Wright et al. 1986; Fujino et al. 1995; Leikauf et al. 1995; Luoto et al. 1997],

— affect mediator release [Morimoto et al. 1993; Ljungman et al.1994; Fujino et al. 1995; Leikauf et al. 1995; Hill et al. 1996; Cullen et al. 1997; Gilmour et al. 1997; Luoto et al. 1997; Wang et al. 1999],

— may decrease cell viability and inhibit proliferation [Yegles et al. 1995; Okayasu et al. 1999; Hart et al. 1992],

— affect cell viability and proliferation [Hart et al. 1992], and

— may induce free radicals, micronuclei, polynuclei, chromosomal breakage, and hyperdiploid cells [Brown et al. 1998; Dopp et al. 1997; Hart et al. 1992].

■ Exposure monitoring results indicate that airborne fibers measured in both the manufacturing and end-use sectors of the RCF industry have dimensions that fall within the thoracic and respirable size ranges [Esmen et al. 1979; Lockey et al. 1990; Cheng et al. 1992].

■ Epidemiologic studies of workers in the RCF manufacturing industry report an association between increased exposures to airborne fibers and the occurrence of pleural plaques, other radiographic abnormalities, respiratory symptoms, decreased pulmonary function, and eye and skin irritation [Lemasters et al. 1994, 1998; Lockey et al. 1996; Trethowan et al. 1995; Burge et al. 1995]. Current occupational exposures to RCFs have not been linked to decreases in pulmonary function of workers [Lockey et al. 1998].

■ Worker exposure to airborne fiber in the RCF industry over the past 20 years or more have decreased substantially, reportedly as the result of increased hazard awareness and the design and implementation of engineering controls [Rice et al. 1997; Maxim et al. 1997].

These observations warrant concern for the continued control and reduction of occupational exposures to airborne RCFs.