Appendix B

Functional Job Categories for RCF Workers

Table B–1. Functional job categories for RCF workers

Functional category	Definition	General examples	Additional comments
Fiber manufacturing	The production or manufacture of RCF bulk or blanket, except in a supervisory capacity. Includes all job functions on the production line, from mixing the raw ingredients to packaging the finished product (bulk or blanket) at the end of the line.	Raw materials, furnace man, furnace operator, or assistant furnace operator Production worker or relief Blanket line Working leader Needler Slit/cut/pack Line utility Utility operator Chopper operator End of line, bagging of bulk RCF End of line trimming, rolling, and packaging of RCF blanket	None to date
Finishing	Cutting or machining RCF materials after fiber manufacture. Hand or power tools may be used in finishing operations.	Operating die stamp on RCF blanket or paper except for automotive applications Sawing, slotting, trimming, or filing casting tips or riser sleeves Cutting blanket for duct wrap	Working in an area where finishing is taking place but not personally working with RCFs unless in a supervisory capacity or in other *auxiliary operations*.

(Continued)

Adapted from Maxim et al. 1997.

Table B–1 (Continued). Functional job categories for RCF workers

Functional category	Definition	General examples	Additional comments
Finishing (Continued)		Cutting or trimming RCF board or other vacuum-formed RCF material capacity Sanding RCF board or other vacuum-formed RCF material Loading sander Off-line cutting and tandem rerolling and/or repackaging of RCF blanket Cutting or trimming RCF modules for use in appliances Milling or routing RCF board or other vacuum-formed RCF material Off-site cutting of batten strips from RCF blanket	EXAMPLE: Unloading dry forms from the drying oven and taking them to the finishing area for final shaping, or packaging shapes immediately after finishing would be considered finishing. However, unloading dry forms from an oven and taking them to be packaged, or packaging shapes that come directly from the drying oven would be considered *auxiliary operations*.
Installation	Building or manufacturing industrial furnaces or boilers, refinery or petrochemical plant equipment, kilns, foundries, electric power generators, and industrial incinerators at end user locations. Includes furnace maintenance. Does not include factory manufacture of industrial furnace components.	Installing hardware or modules On-site cutting (trimming) modules to fit Caulking and filling gaps Wrapping molds with RCF Spraying or pumping RCF castable material inside furnace Cutting and installing laid-in blanket	Working inside furnace during the installation of RCF materials, even though not working directly with that material (e.g., a plumber or electrician working inside a furnace during an installation)
Removal	Removal of after-service RCF material from an industrial furnace, etc., that has completed its economic life. Includes the removal of RCF material during furnace maintenance.	Unwrapping and knocking out molds Furnace disassembly Furnace maintenance Cleanup and disposal of removed material	Working inside furnace during the removal of RCF materials, even though not working directly with that material (e.g., a plumber or electrician working inside a furnace during a removal)
Assembly operations	Combining or assembling RCF material with other material (RCF or other), except automotive applications. Includes factory assembly of industrial furnace components.	Laminating Cutting material for modules Encapsulating RCF blanket Unpacking blanket and loading into module folder	

(Continued)

Table B–1 (Continued). Functional job categories for RCF workers

Functional category	Definition	General examples	Additional comments
Assembly operations (Continued)		Installing bands around modules Packaging modules at end of line Trimming modules and installing hardware Assembling appliances Off-site assembly of industrial furnace components (original equipment manufacture) Changing RCF gaskets, etc. in appliances Cutting and assembling material for sound-proofing exhaust ducts Sewing RCF material Stapling RCF material Ball milling or grinding RCF material Mixing RCF putties, compounds, or castables	
Mixing/forming	Wet end production of vacuum-cast shapes, board, and felt	Forming RCF board or shapes Weighing, batching, or mixing materials to be formed Placing wet parts on conveyor Operating mixing machine Felting	Premixing dry materials before adding to mix tank
Auxiliary operations	Jobs in which workers are *passively* exposed to RCFs while performing their normal duties and whose *exposures are not likely to parallel those of workers working directly with RCF materials*. Includes certain jobs in which RCFs may be handled but with small probability of significant exposures (e.g., warehouse worker or person unloading completed parts for packaging).	Moving RCF-wrapped molds into and out of furnace Warehouse duties, including dock work, loading trucks, moving materials Supervising Driving forklift Making cartons to package RCFs at end of line Quality control inspection Packaging dry parts Maintaining or repairing equipment except furnaces	

(Continued)

Table B–1 (Continued). Functional job categories for RCF workers

Functional category	Definition	General examples	Additional comments
Auxiliary operations (Continued)		Cleaning furnaces or plant areas where RCFs are used Removing vacuum-formed parts from oven and/or packaging them (no finishing)	
Other (not elsewhere classisfied)	All duties performed in the production of RCF paper, textiles, and automotive components or other industry sectors not covered in any of the foregoing categories. Also, exposures that cannot reasonably be included in the categories listed above (i.e., not elsewhere classified). Industrial hygienist personnel should explain tasks and industry sectors as fully as possible for observations in this category.	Diecutting parts for automotive airbag filters, gaskets, mufflers, or catalytic converters Wrapping substrate for catalytic converter Operating former to make roving Operating tape loom Operating carding machine Papermaking	Wrapping RCF blanket around a hot weld so the weld may cool without stress between the hot seam or joint and the cooler surrounding metal (not elsewhere classified)

Appendix C

Cellular and Molecular Effects of RCFs (In Vitro Studies)

The cellular and molecular effects of RCF exposures have been studied with two different objectives. One purpose of these in vitro studies is to provide a quicker, less expensive, and more controlled alternative to animal toxicity testing. These experiments, which strive to act as screening tests or alternatives to animal studies, are best interpreted by comparing their results with those of in vivo experiments. The second objective of in vitro studies is to provide data that may help to explain the pathogenesis and mechanisms of action of RCFs at the cellular and molecular levels. These cytotoxicity and genotoxicity studies are best interpreted by comparing the effects of RCFs with those of other SVFs and asbestos fibers. In vitro studies serve as an important complement to animal studies and provide important tools for studying the molecular mechanisms of fibers. It is not yet possible to use these data in the derivation of an REL.

Drawing strong conclusions relevant to human health based on these in vitro studies is impossible. One point to consider when reviewing these data is the relevance of the cell types studied. Many studies to date have examined the effects of RCFs on rodent cell lines. The cytotoxic effects of RCFs may vary with cell size, volume, and lineage. Effects observed in the cells from organs other than the lung or effects in species other than the human may not be similar to those elicited with human pulmonary cells. The human alveolar macrophage has a volume several times greater than that of the rat alveolar macrophage [Kromback et al. 1997]. Macrophage size and volume may affect (1) the size range of fibers that can be phagocytized, dissolved, and cleared by the lungs and (2) the resulting pathogenicity of the fiber. Even the use of a human lung cell line does not guarantee that in vitro results will be directly applicable to the intact human response. The in vivo integration of stimuli from the nervous, hormonal, and cardiovascular systems cannot be reproduced in vitro.

Another point to consider when reviewing these data is the number and definitions of variables used in different studies. Variables include differences in fiber type, fiber length, fiber dose, cell type, and length of exposure tested, among others. Disparate results between studies make strong conclusions from in vitro studies difficult. At the same time, these studies may provide important data regarding the mechanism of action of RCFs that would not be obtainable in other testing venues.

RCFs may exert their effects on pulmonary target cells via direct or indirect mechanisms. Direct mechanisms are the resultant effects when fibers come in direct physical contact with cells. Direct cytotoxic effects of RCFs include effects on cell viability, responses, and proliferation.

Indirect cellular effects of RCFs involve the interaction of fibers with inflammatory cells that may be activated to produce inflammatory mediators. These mediators may affect target cells directly or may attract other cells that act on target cells. An inflammatory cell type often used in RCF in vitro studies is the pulmonary macrophage. Pulmonary macrophages are the first line of defense against inhaled material that deposits in the alveoli, and among functions, they attempt to phagocytize particles deposited in the lung. Effects of RCF exposure on macrophages and other inflammatory cells are assessed by the measurement of inflammatory mediator release in vitro.

Three important groups of inflammatory mediators are cytokines, ROS, and lipid mediators (prostaglandins and leukotrienes). Some of the cytokines that have been implicated in the inflammatory process include TNF and interleukins (ILs). TNF and many ILs stimulate the deposition of fibroblast collagen, an initial step in fibrosis, and prostaglandins (PG)s inhibit these effects. ROS include hydroxyl radicals, hydrogen peroxide, and superoxide anion radicals. Oxidative stress occurs when the ROS level in a cell exceeds its antioxidant level. Oxidative stress may result in damage to deoxyribo nucelic acid (DNA), lipids, and proteins.

Either direct or indirect effects of RCFs may result in genotoxic effects on pulmonary target cells. Changes in the genetic material may be important in tumor development [Solomon et al. 1991]. Genotoxic effects may be assessed through the analysis of chromosome changes or alterations in gene expression following exposure to RCFs.

The following summary of RCF in vitro studies examines their direct effects on cell proliferation and viability and indirect effects via release of TNF, ROS, and other inflammatory mediators. The genotoxic effects of RCFs are also examined and summarized. Table C–1 describes RCF cytotoxicity studies involving their direct effects on cells. Table C–2 describes RCF cytotoxicity studies involving the release of mediators. Table C–3 summarizes RCF genotoxic studies.

C.1 Direct Cytotoxic Effects of RCFs

RCFs may have a direct cytotoxic effect on target cells. Measurements of cell viability and cell proliferation are both indications of cytotoxic effects. Cell viability can be assessed through the detection of enzymes released by cells or dyes taken up by cells that indicate altered cell membrane integrity or permeability. Measurement of cytoplasmic LDH and trypan blue exclusion are two methods used to assess cell viability. LDH is a cytoplasmic enzyme; its release indicates plasma membrane damage. Trypan blue is a dye that can only penetrate damaged cell membranes. β-glucuronidase is a lysosomal enzyme, it assesses lysosomal permeability and membrane viability. It may also be released when alveolar macrophages are activated by frustrated phagocytosis. The cytotoxic effects of RCFs on rat pleural mesothelial cells, porcine aortic endothelial cells, human-hamster hybrid (A_L) cells, human macrophages, macrophage-like P388D1 cells, and human alveolar epithelial cells are summarized in Table C–1 and C–2 and in the text below.

Luoto et al. [1997] evaluated the effects of RCFs, quartz, and several MMVFs on LDH levels in rat alveolar macrophages and hemolysis in sheep erythrocytes. RCF1, RCF2, RCF3, and RCF4 at 1.0 mg/ml induced a lower release of LDH (less than 20% of control) from rat alveolar macrophages compared with quartz (approximately 40% of control) [Luoto et al. 1997]. RCF1 stimulated the lowest amount of

LDH release (less than 10% of control), lower even than TiO_2 (approximately 15% of control). RCF1, RCF2, RCF3, RCF4, MMVF10, MMVF11, MMVF21, and MMVF22 at 0.5, 2.5, and 5.0 mg/ml induced a dose-dependent increase in sheep erythrocyte hemolysis. RCF1 and RCF3 induced slightly more hemolysis than other MMVFs. The hemolytic activity of MMVFs was similar to that of TiO_2, and much less than that of quartz.

At doses of 100, 300, and 1,000 µg/ml RCFs (unspecified type), an increased release of LDH was induced from rat macrophages [Leikauf et al. 1995]. At equivalent gravimetric doses of 1,000 µg/ml, the effects of RCFs were much less than those of silica. Ceramic fibers (unspecified type) at 50 µg/ml induced no difference in LDH levels compared with negative controls in rat alveolar macrophages [Fujino et al. 1995]. Chrysotile, crocidolite, amosite, and anthophyllite asbestos all induced significant increases in LDH and β-glucuronidase levels. Ceramic fibers also induced a significant increase in β-glucuronidase but much less than that induced by each of the asbestos fiber types.

In the permanent macrophage-like cell line P388D1, an elutriated ceramic fiber (unspecified type) at 10 or 50 µg/ml after 24 or 48 hr had no significant effect on cell viability as measured by the trypan blue assay [Wright et al. 1986]. The elutriation process used for this experiment provided mainly respirable fibers. All other fibers examined, excluding short-fiber amosite, reduced viability. Although the specific data on the effect of exposure to fibers on enzyme release was not presented, an association between decreasing cell viability and increasing loss of intracellular glucosaminidase and LDH was reported under most conditions investigated. Cytotoxicity was correlated with fiber lengths greater than 8 µm when all fiber types were combined.

The effect of several fibers on the viability of rat pleural mesothelial cells was investigated [Yegles et al. 1995]. On a per weight basis, the rank order of cytotoxicity was National Institute for Environmental Health Sciences (NIEHS) chrysotile, RCF3, MMVF10 and RCF1, Calidria chrysotile, RCF4, and all others. Based on the total number of fibers, the rank order of cytotoxicity was RCF3, MMVF10, RCF1, RCF4, MMVF11, NIEHS chrysotile, amosite, and all others. Cytotoxicity was dependent on fiber dimensions as the longest (RCF3, MMVF10, RCF1, MMVF11) or thickest (RCF4, RCF1, MMVF11, RCF3) fibers were the most cytotoxic.

RCF1, RCF2, RCF3, and RCF4 were found to inhibit the proliferation and colony-forming efficiency of Chinese hamster ovary cells in vitro [Hart et al. 1992]. The inhibition was concentration-dependent. RCF4 was least cytotoxic, RCF2 was intermediate, and RCF1 and RCF3 were the most cytotoxic. A correlation existed between average fiber length and toxicity, with the shortest fibers being least cytotoxic. LC_{50}s for the RCF ranged from 10 to 30 µg/cm^2. In each assay, the RCFs were less cytotoxic than those of the positive controls of crocidolite (LC_{50}=5 µg/cm^2) and chrysotile (LC_{50}=1 µg/cm^2) asbestos.

At 0 to 80 µg/cm^2 RCF1, tremolite, and erionite were significantly less cytotoxic to human-hamster hybrid A_L cells than chrysotile as determined by the surviving fraction of colonies after fiber exposure [Okayasu et al. 1999]. RCF1, crocidolite asbestos, and MMVF10 at 25 µg/cm^2 induced focal necrosis in rat pleural mesothelial cells after 24 hr that became a more obvious necrosis by 72 hr [Janssen et al. 1994]. At 72 hr, the qualitative effects of 25 µg/cm^2 RCF1 were comparable to those of 5 µg/cm^2 crocidolite asbestos. In contrast, minimal necrosis was seen at 25 µg/cm^2 crocidolite asbestos, RCF2, and

MMVF10 fibers in hamster tracheal epithelial cells at 24 hr; no necrosis was present at 72 hr.

RCF1, RCF2, RCF3, and RCF4 as well as asbestos and other fibers had a dose-dependent effect on cytotoxicity, as measured by cell detachment, in the human alveolar epithelial cell line A549 [Cullen et al. 1997]. Cell detachment is associated with epithelial damage, an important step in the inflammatory process. These cells are a primary target of inhaled fibers. When equivalent doses (10, 25, 50, and 100 µg/ml) were tested with various fibers, all RCFs had a less significant effect than both crocidolite and amosite asbestos. When the dose data were adjusted for the number of fibers, RCF1, RCF2, and RCF3 were more cytotoxic than RCF4 and crocidolite.

In an assay determining the ability of fibers to induce an increase in the diameter of human A549 cells, an elutriated ceramic fiber (unspecified type) had a midrange of activity when compared with 12 other fibers [Brown et al. 1986]. It was more active than most varieties of amosite tested (but not UICC amosite) but less active than the chrysotile fibers. An association was found between increasing length and assay activity. When these same fiber samples were tested for colony inhibition in V79/4 Chinese hamster lung fibroblasts, the ceramic fiber had even less effect than the TiO_2 control. Analysis of all fibers upheld the association between increasing length and increased activity. In both assays, fiber diameter was not related to activity in most cases.

Chrysotile asbestos at 10 µg/cm^2 and crocidolite asbestos at 5 µg/cm^2 altered porcine aortic endothelial cell morphology and increased neutrophil adherence [Treadwell et al. 1996]. RCF1 fibers at 10 µg/cm^2 did not change cell morphology or increase neutrophil binding.

These studies suggest that RCFs may have some similar direct cytotoxic effects to asbestos. They are capable of inducing enzyme release and cell hemolysis. They may decrease cell viability and inhibit proliferation. In most studies, the effects of RCFs are much less pronounced than the effects of asbestos at similar gravimetric concentrations. Fiber length was demonstrated to be an important factor in determining the cell responses in many studies.

C.2 Indirect Effects of RCFs: Effects on Inflammatory Cells

In addition to direct effects on target cells, RCFs may have indirect mechanisms of action by acting on inflammatory cells. Inflammatory cells, such as pulmonary macrophages, may respond to fiber exposure by releasing inflammatory mediators that initiate the process of pulmonary inflammation and fibrosis. Cytokines and ROS are among the inflammatory mediators released. Many studies, summarized below and in Table C–2, have investigated this link between fiber exposure and mediator release to try to elucidate the mechanism of action of RCFs. Cytokines are a class of proteins that are involved in regulating processes such as cell secretion, proliferation, and differentiation. One of the cytokines most commonly analyzed in RCF cytotoxicity studies is TNF. TNF has been implicated in silica- and asbestos-induced pulmonary fibrosis [Piguet et al. 1990; Lemaire and Ouellet 1996]. TNF and many ILs are associated with collagen deposition (an initial stage of fibrosis), and PGs inhibit these effects. Experiments on the effects of RCF exposure on TNF production in various cell types have had differing results.

TNF secretion has been associated with exposure to asbestos both in vitro and in vivo

[Perkins et al. 1993]. In vitro incubation of human alveolar macrophages from normal volunteers with 25 µg/ml chrysotile asbestos resulted in increased levels of TNF secretion. Alveolar macrophages from 6 human subjects occupationally exposed to asbestos for more than 10 years secreted increased amounts of the cytokines TNF, IL–6, PGE_2, and IL–1ß in vitro. Five human subjects occupationally exposed for less than 10 years did not show increases in these cytokines. The two exposure groups were matched for age, smoking history, and diagnosis. The increased TNF secretion in both in vitro and chronic in vivo asbestos-exposed conditions suggests its importance in the response of the lung to fiber exposure, although the small exposure group sizes warrant caution in drawing strong conclusions.

When equal numbers (8.2×10^6) of various fiber types, including RCF1, RCF2, RCF3, and RCF4, were incubated separately with rat alveolar macrophages, silicon carbide whiskers, amosite, and crocidolite asbestos stimulated the highest TNF release [Cullen et al. 1997]. RCF1, RCF2, RCF3, and RCF4 showed no significant increase in TNF release compared with control.

In contrast, ceramic fibers (unspecified type) at 50 µg/ml (1.72×10^5 f) significantly increased TNF release compared with controls in rat alveolar macrophages [Fujino et al. 1995]. Potassium octatitanate whisker, chrysotile, and crocidolite asbestos induced the greatest TNF release. Alveolar macrophages exposed to either 300 or 1,000 µg/ml RCFs or 1,000 µg/ml asbestos showed a significant increase in TNF production [Leikauf et al. 1995]. At 300 µg/ml RCFs, a significant elevation occurred in leukotriene B_4. At 1,000 µg/ml RCFs, significant elevations occurred in leukotriene B_4 and prostaglandin E_2. Levels induced at lower doses were not different from controls. At equivalent doses, the effect on the levels of all mediators measured was greater after asbestos exposure than after RCF exposure.

Chrysotile A, chrysotile B, crocidolite, MMVF21, RCF1, and silicon carbide at 100 µg/ml caused a significantly increased synthesis of TNF mRNA after 90 minutes of incubation with rat alveolar macrophages [Ljungman et al.1994]. After 4 hr of incubation, chrysotile A still had a significantly increased TNF mRNA production, and all other fibers were at baseline concentrations. None of the fibers studied increased TNF release at 90 minutes. However, an increased TNF bioactivity occurred after 4 hr of incubation with chrysotile A, chrysotile B, crocidolite, or MMVF21. RCF1 at 100 µg/ml did not increase TNF production under these conditions.

Chrysotile asbestos and alumina silicate ceramic fibers increased in vitro alveolar macrophage TNF production in rats exposed to cigarette smoke in vivo and in rats unexposed to smoke [Morimoto et al. 1993]. Asbestos at 50 and 100 µg/ml induced a significantly greater TNF release in rats exposed to cigarette smoke versus unexposed rats. No significant differences were found between groups at all doses of RCF fibers tested (25, 50 and 100 µg/ml). RCF exposure, in contrast to chrysotile, did not have a significant synergistic effect with cigarette smoke exposure.

In addition to the cytokines such as TNF, another group of inflammatory mediators that has been studied in vitro are the ROS. These mediators, also referred to as reactive oxygen metabolites (ROMs) are normally produced during the process of cellular aerobic metabolism and in phagocytic cells in response to particle exposure. One molecular effect of asbestos exposure has been demonstrated to be the induction of ROS [Kamp et al. 1992]. Oxidative stress occurs when the ROS level in a cell exceeds the antioxidant level. ROS may result in

damage to DNA, lipids and proteins and have been implicated in having a role in carcinogenesis [Klaunig et al. 1998; Vallyathan et al. 1998]. This research has suggested that free radical activity may be involved in the pathogenesis of fiber-induced lung disease.

The ability of RCFs to induce the release of free radicals has been studied in rodent alveolar macrophages. RF1 and RF2 (Japanese ceramic fibers) at 200 μg/ml induced a significant production of superoxide anion and a significant increase in intracellular free calcium in guinea pig alveolar macrophages [Wang et al. 1999]. Both superoxide anion and increased intracellular calcium are associated with oxidative stress. Superoxide anions may generate hydrogen peroxide and hydroxyl radical, classified as ROS or free radicals. RF2 exposure resulted in a significant depletion of glutathione. Glutathione is a cellular antioxidant that protects cells against oxidative stress; depletion of glutathione is associated with oxidative stress. The RFs did not affect hydrogen peroxide production. In each test, the effects of chrysotile were significantly greater than those of the RFs.

RCF1, MMVF10, and amosite asbestos at 8.24×10^6 f/ml induced a significant depletion of intracellular glutathione in rat alveolar macrophages [Gilmour et al. 1997]. RCF1 had similar effects to amosite asbestos, whereas MMVF10 caused the greatest depletion of glutathione.

RCF1, RCF2, and RCF3 induced a greater production of ROMs in human polymorphonuclear cell cultures than RCF4 and chrysotile [Luoto et al. 1997]. A dose-dependent production of ROMs was seen in all RCFs and other MMVFs tested from 25 to 500 μg/ml. Quartz had a greater effect on ROM production than all fibers tested.

RCF1 had a high binding capacity for rat immunoglobulin (IgG), a normal component of lung lining fluid [Hill et al. 1996]. At doses >100 μg RCF1, fibers coated with IgG induced a significantly increased superoxide anion release. This supports the premise that lung lining fluid and other substances that fibers are exposed to in vivo may significantly alter the effect of fibers on cells. IgG-coated long fiber amosite asbestos, in spite of a poor binding affinity for IgG, induced a comparable superoxide anion release response to that of coated RCF1.

Brown et al. [1999] investigated the ability of RCF1, amosite asbestos, silicon carbide, MMVF10, Cole 100/475 glass fiber, and RCF4 to cause translocation of the transcription factor NF-κB to the nucleus in A549 lung epithelial cells. RCF1, amosite asbestos, and silicon carbide produced a significant dose-dependent translocation of NF-κB to the nucleus; the other fibers tested did not. Equal fiber numbers were tested.

These cytotoxicity studies indicate that RCFs may share some aspects of their mechanism of action with asbestos. They both affect the production of TNF and ROS as well as cell viability and proliferation. The effects of RCFs have usually been less pronounced than those of asbestos. Results of in vitro studies are difficult to compare, even within studies of different fiber types, because of different study designs, different fiber concentrations and characteristics, and different endpoints.

C.3 Genotoxic Effects of RCFs

In addition to research assessing the cytotoxicity of RCFs, studies have also assessed the genotoxicity of RCFs. Most genotoxicity assays assess changes in or damage to genetic material. Methods that have been used to investigate the genotoxicity of fibers include cell-free or in

vitro cell systems investigating DNA damage, studies of aneuploidy or polyploidy, studies of chromosome damage or mutation, gene mutation assays, and investigations of cell growth regulation [Jaurand 1997]. Several studies, described below and in Table C–3, have examined the ability of RCFs to produce genotoxic changes in comparison with asbestos.

Several fibers, including RCF1 and RCF4, were assayed for free radical generating activity using a DNA assay and a salicylate assay [Brown et al.1998]. The DNA plasmid assay showed only amosite asbestos to have free radical activity. The salicylate assay showed amosite as well as RCF1 to have free radical activity. Coating the fibers with lung surfactant decreased their hydroxyl radical generation. Differences in RCF1 results in the two assays were proposed to be a result of increased iron release from RCF1 in the salicylate assay. An iron chelator inhibited the RCF hydroxylation of salicylate. RCF4 showed no free radical activity.

When equal fiber numbers were compared, RCF1, RCF2, RCF3, and RCF4 had minimal free- radical-generating activity on plasmid DNA compared with crocidolite and amosite asbestos [Gilmour et al. 1995]. RCFs and other MMVF produced a small but insignificant amount of DNA damage. This damage was mediated by hydroxyl radicals. No correlation was found between iron content and free radical generation. At 9.3×10^5 fibers per assay, amosite produced substantial free radical damage to plasmid DNA [Gilmour et al. 1997]. Amosite significantly upregulated the transcription factors AP–1 and NFkB; RCF1 had a much smaller effect on AP–1 upregulation only.

SVFs, including ceramic fibers (unspecified), were reported to form hydroxyl radicals based on the formation of the DNA adduct 8-hydroxydeoxyguanosine (8-OH-dG) from 2-deoxyguanosine (dG) in calf thymus DNA and solution [Leanderson et al. 1989; Leanderson and Tagesson 1989]. Ceramic and glasswool fibers had poor hydroxylating capabilities relative to rockwool and slag wool fibers [Leanderson et al. 1989]. Hydroxyl radical scavengers, such as dimethyl sulfoxide, decreased the hydroxylation. Compounds that increase hydroxyl radical formation, such as hydrogen peroxide, increased hydroxylation. Rockwool in combination with cigarette smoke condensate caused a synergistic increase in 8-OH-dG formation; ceramic and glasswool fibers did not have synergistic effects with cigarette smoke [Leanderson and Tagesson 1989].

RCF1, RCF2, RCF3, and RCF4 induced nuclear abnormalities, including micronuclei and polynuclei, in Chinese hamster ovary cells [Hart et al. 1992]. Micronuclei may form when chromosomes or fragments of chromosomes are separated during mitosis. Polynuclei may arise when cytokinesis fails after mitosis. The incidence of micronuclei and polynuclei after exposure to 20 $\mu g/cm^2$ RCF was from 22% to 33%. At 5 $\mu g/cm^2$, chrysotile and crocidolite induced nuclear abnormalities of 49% and 28%, respectively.

Amosite, chrysotile, and crocidolite asbestos, and ceramic fibers caused a significant increase in micronuclei in human amniotic fluid cells [Dopp et al. 1997]. The response was dose-dependent with asbestos fiber exposure but not with ceramic fiber exposure. Significant increases in chromosomal breakage and hyperdiploid cells were noted after asbestos and ceramic fiber exposure.

RCF1, RCF3, and RCF4 did not induce anaphase aberrations in rat pleural mesothelial cells [Yegles et al. 1995]. Of all fibers tested, UICC chrysotile was the most genotoxic on the basis of weight, number of fibers with a length >4 μm and number of fibers corresponding

to Stanton's and Pott's criteria [Stanton et al. 1981; Pott et al. 1987].

The effect of fibers on the mRNA levels of c-fos and c-jun proto-oncogenes and ornithine decarboxylase (ODC) in hamster tracheal epithelial (HTE) cells and rodent pleural mesothelial (RPM) cells were examined [Janssen et al. 1994]. ODC is a rate-limiting enzyme in the synthesis of compounds involved in cell proliferation and tumor promotion, the polyamines. In HTE cells, crocidolite induced a significant dose-dependent increase in levels of c-jun and ODC mRNA but not c-fos mRNA. RCF1 induced only small nondose-dependent increases in ODC mRNA levels. In RPM cells, crocidolite fibers at 2.5 μg/cm^2 significantly elevated levels of c-fos and c-jun mRNA. RCF1 increased proto-oncogene expression at cytotoxic levels of 25 μg/cm^2; no significant effect was seen at concentrations ≤5 μg/cm^2.

RCF1 fibers were nonmutagenic in the human-hamster hybrid cell line A_L [Okayasu et al. 1999]. Chrysotile was a significant inducer of mutations in the same system.

These studies demonstrate that RCFs may share some similar genotoxic mechanisms with asbestos including induction of free radicals, micronuclei, polynuclei, chromosomal breakage, and hyperdiploid cells. Other studies have demonstrated that, using certain methods and doses, RCFs did not induce anaphase aberrations and induced proto-oncogene expression only at cytotoxic concentrations. RCFs were nonmutagenic in human-hamster hybrid cells.

C.4 Discussion of In Vitro Studies

The toxicity of fibers has been attributable to their dose, dimensions, and durability. Any test system that is designed to assess the potential toxicity of fibers must address these factors. Durability is difficult to assess using in vitro studies because of their acute time course. However, in vitro studies provide an opportunity to study the effects of varying doses and dimensions of fibers in a quicker, more efficient method than animal testing. Although they provide important information about mechanism of action, they do not currently provide data that can be extrapolated to occupational risk assessment.

The association between fiber dimension and toxicity has been documented and reviewed [Stanton et al. 1977, 1981; Pott et al. 1987; Warheit 1994]. Fiber length has been correlated with the cytotoxicity of glass fibers [Blake et al. 1998]. Manville code 100 (JM–100) fiber samples of average lengths of 3, 4, 7, 17, and 33 μm were assessed for their effects on LDH activity and rat alveolar macrophage function. The greatest cytotoxicity was reported in the 17 μm and 33 μm samples, indicating that length is an important factor in the toxicity of this fiber. Multiple macrophages were observed attached along the length of long fibers. Relatively short fibers, <20 μm long, were usually phagocytized by one rat alveolar macrophage [Luoto et al. 1994]. Longer fibers were phagocytized by two or more macrophages. Incomplete, or frustrated, phagocytosis may play a role in the increased toxicity of longer fibers. Long fibers (17 μm average length) were a more potent inducer of TNF production and transcription factor activation than shorter fibers (7 μm average length) [Ye et al. 1999]. These studies demonstrate the important role of length in fiber toxicity and suggest that the capacity for macrophage phagocytosis may be a critical factor in determining fiber toxicity. The toxicity of individual fibers of the same type of RCFs may differ according to their size in relation to alveolar macrophages.

Several RCF in vitro studies reported a direct association between a longer fiber length and greater cytotoxicity. Hart et al. [1992] reported the shortest fibers to be the least cytotoxic. Brown et al. [1986] reported an association between length and cytotoxic activity but not between diameter and activity. Wright et al. [1986] reported that cytotoxicity was correlated with fibers >8 μm length. Yegles et al. [1995] reported that the longest and thickest fibers were the most cytotoxic. The four most cytotoxic fibers had GM lengths ≥13 μm and GM diameters >0.5 μm. The production of abnormal anaphases and telophases was associated with Stanton fibers with a length >8 μm and diameter ≤0.25 μm. Hart et al. [1994] reported that cytotoxicity increased with fiber length up to 20 μm. All of these studies demonstrate the importance of fiber dimensions on cytotoxicity. Other studies have not reported the length distribution of fiber samples used. When studies are done with RCFs for which specific lengths are assessed for cytotoxicity (such as has been done with glass fibers) [Blake et al. 1998], it will be possible to determine the strength of the association between RCF fiber length and toxicity and determine whether a threshold length exists above which toxicity increases steeply.

In addition to providing data on the correlation between fiber length and toxicity, in vitro studies have provided data on the relative toxicity of RCFs compared with asbestos. Uncertainties exist in the interpretation of these studies because of differences in fiber doses, dimensions, and durabilities. RCFs do appear to share some similar mechanisms of action with asbestos. (See references in Tables C–1, C–2, and C–3.) They have similar direct and indirect effects on cells and alter gene function in similar ways. They are capable of inducing enzyme release and cell hemolysis. They may decrease cell viability and inhibit proliferation. They both affect the production of tumor necrosis factor and ROS, and affect cell viability and proliferation. They induce necrosis in rat pleural mesothelial cells. They also may induce free radicals, micronuclei, polynuclei, chromosomal breakage, and hyperdiploid cells in vitro.

In vitro studies also provide an excellent opportunity for investigating the pathogenesis of RCF. However, comparisons are difficult to make between in vitro studies because of differences in fiber doses, dimensions, preparations, and compositions. Important information, such as fiber length distribution, is not always determined. Even when comparable fibers are studied, the cell line or conditions under which they are tested may vary. Much of the research to date has been done in rodent cell lines and in cells that are not related to the primary target organ. In vitro studies using human pulmonary cell lines should provide pathogenesis data most relevant to human health risk assessment.

Short-term in vitro studies cannot take into account the influence of fiber dissolution and fiber compositional changes that may occur over time. In an in vivo exposure, fibers are continually modified physically, chemically, and structurally by components of the lung environment. This complex set of conditions is difficult to recreate in vitro. Just as it is unlikely that only one factor will be an accurate predictor of fiber toxicity, it is much more unlikely that any one in vitro test will be able to predict fiber toxicity. Best results are obtained by toxicity assessment in several in vitro tests and in comparison with in vivo results. In vitro studies provide an excellent opportunity to investigate factors important to fiber toxicity such as dose, dimension, surface area, and physicochemical composition. They provide the ability to obtain information that is an important supplement to the data of chronic inhalation studies. They do not currently provide information that can be directly applied to human health risk assessment and the development of occupational exposure limits.

Table C–1. In vitro cytotoxicity of RCFs:* direct effects on cells

Reference	Cell line and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Brown et al. [1986]	A549 cells Cell diameter V79/4 Chinese hamster lung fibroblasts Colony inhibition	Elutriated (E) ceramic (unspecified) Titanium dioxide Quartz Short fiber amosite UICC crocidolite E factory amosite E UICC crocidolite E brucite UICC amosite Superfine chrysotile E UICC anthophyllite UICC chrysotile A E tremolite E long fiber amosite E UICC chrysotile A	Not reported	Not reported	A549 cells: 25 or 50 μg/ml V79/4 cells: 0, 5, 10, 25, 75, or 100 μg/ml	A549 assay: Chrysotile effect> ceramic effect> most amosites (not UICC amosite). V79/4 assay: Ceramic fiber had no effect. Ceramic fiber had different results in the two assays. Association found between increasing fiber length (all types) and activity in both assays.
Cullen et al. [1997]	Human alveolar epithelial cells Cell detachment	RCF1 RCF2 RCF3 RCF4 Long amosite Crocidolite C100/475 glass 104E glass Silicon carbide 1 Silicon carbide 2 MMVF10 MMVF11 MMVF21 MMVF22	Geometric mean: 10.42 ± 2.66 12.43 ± 2.66 14.99 ± 2.64 6.82 ± 2.00 3.03 ± 2.86 4.96 ± 2.57 2.88 ± 2.62 3.50 ± 2.17 8.73 ± 2.25 Not done 23.91 ± 2.39 14.21 ± 2.64 15.66 ± 2.76 13.67 ± 2.34	Geometric mean: 0.79 ± 2.07 0.84 ± 2.01 0.71 ± 2.12 0.94 ± 1.71 0.26 ± 1.75 0.15 ± 1.53 0.22 ±1.85 0.25 ± 1.6 0.47 ± 1.39 Not done 1.13 ± 1.90 0.57 ± 2.01 0.81 ± 1.76 0.89 ± 1.78	10, 25, 50, or 100 μg/ml	At equivalent doses, all RCFs had less effect than crocidolite and amosite asbestos. When adjusted for equivalent fiber numbers, crocidolite, RCF4, MVF11, and amosite were least cytotoxic; RCF1, RCF2, and RCF3 were more cytotoxic than crocidolite and amosite.

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Table C–1 (Continued). In vitro cytotoxicity of RCFs:* direct effects on cells

Reference	Cell line and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Fujino et al. [1995]	Rat alveolar macrophages LDH β-glucuronidase	Ceramic Glass Potassium octatitanate Magnesium sulfate (long) Magnesium sulfate (short) Chrysotile asbestos Crocidolite asbestos Amosite Anthophyllite Erionite	Geometric mean: 29.5 ± 3.1 12.8 ± 3.0 2.8 ± 2.0 12.0 ± 2.3 4.9 ± 2.1 0.7 ± 1.9 1.3 ± 2.3 2.7 ± 2.5 2.5 ± 3.5 1.4 ± 2.0	Geometric mean: 1.92 ± 2.9 0.54 ± 2.2 0.41 ± 1.5 0.44 ± 1.6 0.31 ± 1.5 0.085 ± 1.4 0.20 ± 1.5 0.32 ± 1.8 0.36 ± 2.3 0.21 ± 1.6	50 μg/ml	LDH: Ceramic fiber, magnesium sulfate whiskers, and erionite were not different from control; all others significantly increased levels. β-glucuronidase: All fibers caused a significant increase compared with control; chrysotile caused the highest release.
Hart et al. [1992]	Chinese hamster ovary cells Cell proliferation Colony formation	RCF1 RCF2 RCF3 RCF4 UICC Crocidolite UICC Chrysotile	Geometric mean: 21.5 ± 16.12 16.7 ± 15.03 24.3 ± 18.82 9.2 ± 7.08 1.8 ± 1.94 1.65 ± 1.83	Geometric mean: 1.03 ± 0.73 1.11 ± 0.82 1.22 ± 0.98 1.43 ± 0.79 0.21 ± 0.12 0.12 ± 0.07	RCF1–4: 0, 5, 10, or 20 μg/ml Crocidolite: 0 or 5 μg/ml Chrysotile: 0, 1, 2, or 5 μg/ml	RCFs induced a concentration-dependent decrease in colony formation and cell proliferation. LC_{50}: RCFs 10–30 μg/cm² Crocidolite 5 μg/cm² Chrysotile 1 μg/cm² RCF cytotoxicity: RCF1 and RCF3 are most cytotoxic; RCF2 is intermediate; RCF4 is least cytotoxic.
Leikauf et al. [1995]	Rat alveolar macrophages LDH	RCF Crocidolite asbestos Silica Titanium dioxide	Median: 4.9 2.5 Not available Not available	Median: 0.59 0.28 0.88 0.18	100, 300, 1,000 μg/ml	RCFs induced moderate increases in LDH levels. % of control: RCFs 100 μg/ml—158% RCFs 300 μg/ml—174% RCFs 1,000 μg/ml—295% Silica 1,000 μg/ml—896%

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Table C–1 (Continued). In vitro cytotoxicity of RCFs:* direct effects on cells

Reference	Cell line and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Luoto et al. [1997]	Sheep erythrocytes Hemolysis Rat alveolar macrophages LDH	RCF1 RCF2 RCF3 RCF4 MMVF10 MMVF11 MMVF21 MMVF22	Mean: 21.29 ± 17.42 20.18 ± 19.63 25.66 ± 25.74 10.34 ± 11.59 23.21 ± 15.57 15.65 ± 13.31 26.02 ± 23.10 20.75 ± 20.52	Mean: 1.30 ± 0.72 1.39 ± 0.98 1.37 ± 0.87 1.33 ± 1.02 1.42 ± 0.78 1.12 ± 0.88 1.18 ± 0.64 0.88 ± 0.45	Hemolysis: 0.5, 2.5, or 5.0 mg/ml LDH: 1.0 mg/ml	Hemolysis: Dose-dependent increase in hemolysis induced by all fibers and dusts. RCF1 and RCF3 were slightly more hemolytic than all other fibers but much less than quartz. LDH: RCF1 induced the least LDH release; MMVF 22 had the greatest fiber-induced LDH release; quartz induced the greatest LDH release overall.
Okayasu et al. [1999]	Human-hamster hybrid A_L cells Surviving colonies	UICC chrysotile Tremolite Erionite RCF1	Geometric mean: 1.78 ± 2.3 1.41 ± 2.7 1.31 ± 2.9 13.5 ± 2.7	Geometric mean: 0.12 ± 0.08 0.13 ± 3.43 0.23 ± 2.74 0.95 ± 2.6	0–400 μg/ml 0–80 μg/cm^2	Chrysotile was more cytotoxic than other fibers. Mean lethal doses: Chrysotile ~4 μg/cm^2 RCF1 35 μg/cm^2 Tremolite 40 μg/cm^2 Erionite 42 μg/cm^2
Wright et al. [1986]	P388D1 Trypan blue assay LDH concentrations Glucosaminidase concentrations	UICC crocidolite UICC amosite UICC chrysotile A E UICC crocidolite E UICC amosite E UICC chrysotile A E UICC anthophyllite E ceramic fiber E long fiber amosite Short fiber amosite E tremolite	Cumulative fiber length distributions reported	Almost all fibers measured had diameters less than 3 μm; details not reported	Fiber number in 10^{-10} g ± SD: 112 ± 10 76 ± 6 185 ± 18 109 ± 10 112 ± 14 144 ± 20 169 ± 15 15 ± 0.4 19 ± 1	Trypan blue assay: Ceramic fibers at both doses had the lowest degree of cytotoxicity; all other fibers, excluding short-fiber amosite, reduced viability. Fibers >8 μm were usually most effective in decreasing viability and increasing LDH and glucosaminidase concentrations; individual fiber effects were not reported.

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Table C–1 (Continued). In vitro cytotoxicity of RCFs:* direct effects on cells

Reference	Cell line and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Wright et al. [1986] (Continued)		E brucite SFA chrysotile Titanium dioxide Quartz			44 ± 2.7 22 ± 1.6 152 ± 14 7.4 ± 0.5 Fiber dose: 10 or 50 μg/ml 80 μg/ml 20 μg/ml	
Yegles et al. [1995]	Rat pleural mesothelial cells Cell viability: Mitochondrial integrity		Mean:	Mean:	12.5, 25, 50, 75, or 100 μg/cm^2	Cytotoxicity per weight basis: Chrys NIEHS > RCF3> MMVF10=RCF1> Chrys, calidria > RCF4 > all others Cytotoxicity per total number of fibers: RCF3 > MMVF10 > RCF1 > RCF4 >MMVF11 > Chrys, NIEHS>amosite >all others Fiber length and diameter correlated with cytotoxicity: longest and thickest fibers were most cytotoxic.
		RCF1	19.2 ± 15.0	1.30 ± 0.80		
		RCF3	31.8 ± 36.0	0.74 ± 0.50		
		RCF4	8.9 ± 7.2	1.30 ± 0.60		
		MMVF10	21.5 ± 16.8	0.55 ± 0.50		
		MMVF11	16.7 ± 12.9	1.10 ± 0.90		
		Chrysotile (chrys), UICC	1.7 ± 2.2	0.05 ± 0.04		
		Chrys 445 (Canadian)	2.3 ± 2.3	0.04 ± 0.03		
		Chrys 443 (Canadian)	1.9 ± 1.9	0.04 ± 0.04		
		Chrys, short Canadian	1.6 ± 1.4	0.06 ± 0.08		
		Chrys, superfine Canadian	2.4 ± 3.1	0.04 ± 0.03		
		Chrys, phosphorylated Canadian	4.7 ± 5.2	0.04 ± 0.03		
		Chrys, phosphorylated, milled Canadian	4.7 ± 5.9	0.07 ± 0.09		
		UICC chrys, leached with oxalic acid	2.3 ± 1.8	0.17 ± 0.11		
		Chrys, Calidria	2.8 ± 3.0	0.05 ± 0.04		
		Chrys, NIEHS	4.2 ± 1.2	0.05 ± 0.05		
		Amosite	2.4 ± 1.8	0.31 ± 0.20		
		Crocidolite	2.1 ± 3.6	0.19 ± 0.12		
		Attapulgite	0.8 ± 0.5	0.04 ± 0.03		

*Abbreviations: E=elutriated; LDH=lactate dehydrogenase; MMVF=man-made vitreous fiber; NIEHS=National Institute of Environmental Health Sciences; RCFs=refractory ceramic fibers; SD=standard deviation; UICC=Union Internationale Contre le Cancer.

Table C–2. In vitro cytotoxicity of RCFs:* effects on mediator release

Reference	Cell line and endpoints	Fiber type	Length (µm)	Diameter (µm)	Dose	Results
			Geometric mean:	Geometric mean:	8.2×10^6 fibers	SiC1, SiC2, crocidolite, and
Cullen et al. [1997]	Human alveolar epithelial cells	RCF1	10.42 ± 2.66	0.79 ± 2.07	(>5 µm long)	long amosite stimulated the highest TNF production.
		RCF2	12.43 ± 2.66	0.84 ± 2.01		
		RCF3	14.99 ± 2.64	0.71 ± 2.12		
	TNF	RCF4	6.82 ± 2.00	0.94 ± 1.71		RCF1–RCF4 did not show more TNF activity than in control cultures.
		Long amosite	3.03 ± 2.86	0.26 ± 1.75		
		Crocidolite	4.96 ± 2.57	0.15 ± 1.53		
		C100/475 glass	2.88 ± 2.62	0.22 ±1.85		
		104E glass	3.50 ± 2.17	0.25 ± 1.6		
		SiC1	8.73 ± 2.25	0.47 ± 1.39		
		SiC2	Not done	Not done		
		MMVF10	23.91 ± 2.39	1.13 ± 1.90		
		MMVF11	14.21 ± 2.64	0.57 ± 2.01		
		MMVF21	15.66 ± 2.76	0.81 ± 1.76		
		MMVF22	13.67 ± 2.34	0.89 ± 1.78		
			Geometric mean:	Geometric mean:	50 µg/ml	All fibers significantly in-
Fujino et al. [1995]	Rat alveolar macrophages	Ceramic	29.5 ± 3.1	1.92 ± 2.9		creased TNF production above no-fiber controls; potassium octatitanate caused the highest TNF production.
		Glass	12.8 ± 3.0	0.54 ± 2.2		
		Potassium octatitanate	2.8 ± 2.0	0.41 ± 1.5		
	TNF	Magnesium sulfate (long)	12.0 ± 2.3	0.44 ± 1.6		
		Magnesium sulfate (short)	4.9 ± 2.1	0.31 ± 1.5		
		Chrysotile asbestos	0.7 ± 1.9	0.085 ± 1.4		
		Crocidolite asbestos	1.3 ± 2.3	0.20 ± 1.5		
		Amosite	2.7 ± 2.5	0.32 ± 1.8		
		Anthophylite	2.5 ± 3.5	0.36 ± 2.3		
		Erionite	1.4 ± 2.0	0.21 ± 1.6		

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Table C–2 (Continued). In vitro cytotoxicity of RCFs:* effects on mediator release

Reference	Cell line and endpoints	Fiber type	Length (µm)	Diameter (µm)	Dose	Results
Gilmour et al. [1997]	Rat alveolar macrophages Glutathione	RCF1 Amosite asbestos MMVF10	Not reported	Not reported	8.24×10^6/ml	All fibers significantly lowered intracellular glutathione. MMVF10 caused the greatest decrease. RCF1 and amosite had similar effects.
Hill et al. [1996]	Rat alveolar macrophages Superoxide anion release after coating with rat immunoglobulin (IgG), a normal component of lung lining fluid.	MMVF21 RCF1 LFA asbestos SiC Johns Manville Code 100/475 (glass)	WHO f/µg 5,462 9,015 164,705 70,550 21,742 Percentage length distribution also reported.	Not reported	RCF1, MMVF21: 125 µg–20 mg/ml Code 100/475, SiC:125 µg–1 mg/ml LFA asbestos: 15.6 µg–5 mg/ml	IgG-coated RCF1 fibers and IgG-coated LFA asbestos fibers induced a significantly increased superoxide anion release. Coating of RCF1 fibers at doses >100 µg greatly increased their superoxide production. RCF1 fibers had a high binding affinity for IgG; LFA asbestos did not.
Leikauf et al. [1995]	Rat macrophages TNF LTB_4 PGE_2	RCF (unspecified) Crocidolite asbestos Silica TiO_2	Median: 4.9 2.5 NA NA	Median: 0.59 0.28 0.88 0.18	100, 300, 1,000 µg/ml	TNF production was increased after exposure to 300 and 1,000 µg/ml RCFs. LTB_4 levels were elevated after exposure to 300 or 1,000 µg/ml RCFs; PGE_2 concentrations were elevated after exposure to 1,000 µg/ml RCFs; effects at lower doses were not significant. At equivalent doses, asbestos induced a greater response than RCFs in TNF, LTB_4, or PGE_2 concentrations.

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Table C–2 (Continued). In vitro cytotoxicity of RCFs:* effects on mediator release

Reference	Cell line and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Ljungman et al. [1994]	Rat alveolar macrophages TNF	Crocidolite Chrysotile A Chrysotile B MMVF21 MMVF22 RCF1 SiCwh	Mean: 9.9 ± 7.8 Not reported Not reported 24.6 ± 19.9 21.4 ± 17.6 22.4 ± 19.0 Not reported	Mean: 0.3 ± 0.2 Not reported Not reported 1.3 ± 0.8 1.2 ± 0.7 1.1 ± 0.8 Not reported	100 μg/ml	Chrysotile A, chrysotile B, crocidolite, MMVF21, RCF1, and SiCwh increased TNF mRNA concentrations after 90 minutes; concentrations had returned to baseline after 4 hours in all but chrysotile A. Chrysotile A, chrysotile B, crocidolite, and MMVF21 induced an increase in TNF bioactivity after 4 hours of incubation; RCF1 did not induce a significant increase.
Luoto et al. [1997]	Rat alveolar macrophages ROM	RCF1 RCF2 RCF3 RCF4 MMVF10 MMVF11 MMVF21 MMVF22	Mean: 21.29 ± 17.42 20.18 ± 19.63 25.66 ± 25.74 10.34 ± 11.59 23.21 ± 15.57 15.65 ± 13.31 26.02 ± 23.10 20.75 ± 20.52	Mean: 1.30 ± 0.72 1.39 ± 0.98 1.37 ± 0.87 1.33 ± 1.02 1.42 ± 0.78 1.12 ± 0.88 1.18 ± 0.64 0.88 ± 0.45	25, 50, 100, 200, 400, or 500 μg/ml	All fibers showed a dose-dependent response to ROM production. RCF1, RCF2, or RCF3 exposure resulted in higher ROM production than RCF4 or chrysotile exposure. Quartz had the greatest effect on ROM production.

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Table C–2 (Continued). In vitro cytotoxicity of RCFs:* effects on mediator release

Reference	Cell line and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Morimoto et al. [1993]	Rat alveolar macrophages TNF	Canadian chrysotile Alumina silicate ceramic (Japan)	Not reported	Mass median aerodynamic diameter = 3.1 μm	25, 50, or 100 μg/ml	Both fibers stimulated a dose-dependent TNF production by alveolar macrophages. Chrysotile stimulated greater TNF production than ceramic fibers in cells from rats exposed to cigarette smoke and cells from rats not exposed to smoke. Chrysotile induced higher TNF production in smoke-exposed rats than in controls; ceramic fiber exposure resulted in no significant difference between TNF production in smoke-exposed rats and controls.
Wang et al. [1999]	Guinea pig alveolar macrophages Superoxide anion Hydrogen peroxide GSH Intracellular free calcium	Japan fibrous material : GW1 = glass wool RW1 = rock wool MG1 = micro glass RF1 = refractory ceramic RF2 = refractory ceramic RF3 = refractory mullite PT1 = potassium titanate SC1 = silicon carbide TO1 = titanium oxide WO1 = wollastonite Chrysotile	 20.2 ± 2.58 16.5 ± 2.51 3.0 ± 2.22 12.0 ± 2.36 11.0 ± 1.96 11.0 ± 1.75 6.0 ± 2.04 6.4 ± 2.45 2.1 ± 2.00 10.5 ± 2.03 Not reported	 0.88 ± 3.10 1.80 ± 2.32 0.24 ± 2.35 0.77 ± 2.53 1.10 ± 2.00 2.40 ± 1.37 0.35 ± 1.51 0.30 ± 1.58 0.14 ± 1.53 1.00 ± 1.72 Not reported	200 μg/ml	Chrysotile and all fibers other than WO1 significantly increased superoxide anion production. Chrysotile significantly increased hydrogen peroxide production; RF did not. Chrysotile, RF2, PT1, TO1, SC1, WO1, and MG1 significantly decreased GSH concentration. Chrysotile, RF1, RF2, SC1, TO1, PT1, MG1, and RW1 significantly increased intracellular free calcium. In all tests, chrysotile had greater effects than those of the RCFs.

*Abbreviations: GSH=glutathione; GW1=glass wool; IgG=immunoglobulin; LFA=long fiber amosite; LTB_4=leukotriene B4; MG1=micro glass; MMVF=man-made vitreous fiber; PGE_2=prostaglandin E_2; PT1=potassium titanate; RCFs=refractory ceramic fibers; RF1=refractory ceramic; RF2=refractory ceramic; RF3=refractory mullite; ROM=reactive oxygen metabolites; RW1=rock wool; SC1=silicon carbide in Wang et al. [1999]; SiC=silicon carbide; TNF=tumor necrosis factor; TiO_2=titanium dioxide; TO1=titanium oxide in Wang et al. [1999]; wh=whiskers; WO1=wollastonite.

Table C–3. In vitro genotoxic effects of RCFs*

Reference	Test system and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Brown et al. [1998]	Plasmid oX174RF1 DNA DNA scission Salicylate assay Hydroxyl radical generation		Size distribution >10 >20	Not reported	Plasmid assay: 9.249×10^5 f/20 μl Salicylate assay: 8.24×10^7 f/ml	Plasmid assay: Only amosite had free radical activity. Salicylate assay: Amosite and RCF1 had free radical activity. Coating the fibers with lung surfactant decreased hydroxyl radical activity. An iron chelator inhibited hydroxylation by RCF1.
		Long fiber amosite asbestos	64.75 35.25			
		SiC	60.86 27.6			
		RCF1	77.36 45.27			
		RCF4	59.35 17.96			
		MMVF10	85.24 67.17			
		Code 100/475 glass	50.00 19.32			
Dopp et al. [1997]	Human amniotic fluid cells Micronuclei Hyperdiploidy Chromosomal breakage		Average:	Average:	0.5, 1.0, 5.0, or 10.0 μg/cm²	All fibers caused a significant increase in micronuclei. Dose-dependent response was seen with asbestos but not with ceramic fiber exposure. Asbestos and ceramic fibers induced chromosomal breakage and hyperdiploid cells.
		Amosite asbestos	2.05	0.24		
		Crocidolite asbestos-	1.71	0.25		
		Rhodesian chrysotile asbestos	2.24	0.10		
		Ceramic (unspecified)	12.03	0.90		
		Gypsum				
Gilmour et al. [1995]	Plasmid oX174RF1 DNA Depletion of supercoiled DNA	Short fiber amosite Long fiber amosite Crocidolite asbestos RCF1 RCF2 RCF3 RCF4 MMVF10 MMVF11 MMVF21 MMVF22	Not reported	Not reported	Tested at equal fiber numbers: 6.166×10^5 or 9.249×10^5 or 1.2332×10^6	RCF1, RCF2, RCF3, and RCF4 had a minimal free radical effect compared with asbestos fibers. RCF DNA damage was mediated by hydroxyl radicals but was not associated with iron content.

See footnote at end of table.

(Continued)

Table C–3 (Continued). In vitro genotoxic effects of RCFs*

Reference	Test system and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Gilmour et al. [1997]	Plasmid oX174RF1 DNA Depletion of supercoiled DNA Rat alveolar macrophages Activation of transcription factors	Amosite asbestos MMVF10 RCF1	Not reported	Not reported	Equal fiber numbers per assay. DNA assay: 9.3×10^5 Iron assay: 8.24×10^7/ml	RCF1 and MMVF10 induced significantly less DNA free radical damage than amosite asbestos. Amosite significantly upregulated transcription factors AP–1 and NFkB; RCF1 had a much smaller effect on AP–1 only.
Hart et al. [1992]	Chinese hamster ovary cells Micronuclei induction Polynuclei induction	RCF1 RCF2 RCF3 RCF4 UICC Crocidolite UICC Chrysotile	Mean: 21.5 ± 16.12 16.7 ± 15.03 24.3 ± 18.82 9.2 ± 7.08 1.8 ± 1.94 1.65 ± 1.83	Mean: 1.03 ± 0.73 1.11 ± 0.82 1.22 ± 0.98 1.43 ± 0.79 0.21 ± 0.12 0.12 ± 0.07	RCFs 1–4: 0, 5, 10, or 20 μg/ml Crocidolite: 0 or 5 μg/ml Chrysotile: 0, 1, 2, or 5 μg/ml	Nuclear abnormality incidence: At 20 μg/cm², RCF was 20% to 33%. At 5 μg/cm², crocidolite was 28%. At 5 μg/cm², chrysotile was 49%.
Janssen et al. [1994]	Hamster tracheal epithelial (HTE) cells mRNA concentrations of c-fos, c-jun, and ODC RPM cells mRNA concentrations of c-fos, c-jun, and ODC	Crocidolite Chrysotile MMVF10 RCF1 Polystyrene beads Riebeckite Erionite	Mean: 11.4 1.1 19.8 24.0 — — 6.0	Mean: 0.27 0.08 1.36 1.07 1.05 0.8 0.8	Asbestos: ≤5 μg/cm² All other fibers: 1.25–25 μg/cm²	HTE cells: Crocidolite induced significant dose-dependent concentrations of c-jun and ODC mRNA. RCF1 induced small non-dose-dependent increases in ODC mRNA concentrations only. RPM cells: Crocidolite at 2.5 μg/cm² induced elevated c-fos and c-jun mRNA concentrations. RCF1 at 25 μg/cm² increased c-fos and c-jun mRNA concentrations.

See footnote at end of table.

(Continued)

Table C–3 (Continued). In vitro genotoxic effects of RCFs*

Reference	Test system and endpoints	Fiber type	Length (µm)	Diameter (µm)	Dose	Results
Leanderson et al. [1989]	Calf thymus DNA Hydroxylation of 2-dG to 8–OH–dG dG solution Hydroxylation of dG to 8–OH–dG	European source: Fiber 1 = ceramic Fiber 2 = glass wool Fiber 3 = ceramic Fiber 4 = rock wool Fiber 5 = rock wool Fiber 6 = rock wool Fiber 7 = rock wool Fiber 8 = rock wool Fiber 9 = rock wool Fiber 10 = rock wool Fiber 11 = rock wool Fiber 12 = slag wool Fiber 13 = rock wool Fiber 14 = rock wool Fiber 15 = slag wool Fiber 16 = rock wool	Surface area (m^2/g): 0.95 0.91 1.10 1.30 0.36 0.60 0.73 1.01 1.28 1.16 1.18 1.14 1.30 1.06 0.90 0.62	Not reported	10 mg fiber and 1.0 ml PBS with 0.5 mg DNA or 0.5 mM dG	All fibers caused significant hydroxylation of dG. Glass wool and ceramic fibers had poor hydroxylating capacity relative to rock wools and slag wools.
Leanderson et al. [1989]	Calf thymus DNA Hydroxylation of dG to 8–OH–dG	European source: Rock wool Glass wool Ceramic	Not reported	Not reported	10 mg fiber and/or 300 µl smoke-PBS or 100 µM H_2O_2 in 1.0 ml PBS with 0.5 mg DNA	Ceramic and glass wool fibers caused less DNA hydroxylation than rock wool. Rock wool and cigarette smoke condensate had a synergistic effect on hydroxylation. Ceramic or glass wool fibers and cigarette smoke condensate did not have a synergistic effect on hydroxylation.
Okayasu et al. [1999]	Human-hamster hybrid A_L cells Mutation assay	UICC chrysotile Tremolite Erionite RCF1	Geometric mean: 1.78 ± 2.3 1.41 ± 2.7 1.31 ± 2.9 13.5 ± 2.7	Geometric mean: 0.12 ± 0.08 0.13 ± 3.43 0.23 ± 2.74 0.95 ± 2.6	0–80 µg/cm^2	RCF1 was determined to be nonmutagenic. Chrysotile was the most mutagenic of fibers examined based on fiber concentration.

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(Continued)

Table C–3 (Continued). In vitro genotoxic effects of RCFs*

Reference	Test system and endpoints	Fiber type	Length (μm)	Diameter (μm)	Dose	Results
Yegles et al. [1995]	Rat pleural mesothelial cells Anaphase/telophase aberrations	RCF1	19.2 ± 15.0	1.30 ± 0.80	12.5, 25, 50, 75, or 100 μg/cm^2	UICC chrysotile was the most genotoxic on the basis of weight, number of fibers >4 μm long, and number of fibers corresponding to Stanton's and Pott's criteria. Ceramic and glass fibers did not induce anaphase aberrations.
		RCF3	31.8 ± 36.0	0.74 ± 0.50		
		RCF4	8.9 ± 7.2	1.30 ± 0.60		
		MMVF10	21.5 ± 16.8	0.55 ± 0.50		
		MMVF11	16.7 ± 12.9	1.10 ± 0.90		
		UICC chrysotile	1.7 ± 2.2	0.05 ± 0.04		
		Chrys 445 (Canadian)	2.3 ± 2.3	0.04 ± 0.03		
		Chrys 443 (Canadian)	1.9 ± 1.9	0.04 ± 0.04		
		Chrys, short Canadian	1.6 ± 1.4	0.06 ± 0.08		
		Chrys, superfine Canadian	2.4 ± 3.1	0.04 ± 0.03		
		Chrys, phosphorylated Canadian	4.7 ± 5.2	0.04 ± 0.03		
		Chrys,phosphorylated milled Canadian	4.7 ± 5.9	0.07 ± 0.09		
		UICC chrys, leached with oxalic acid	2.3 ± 1.8	0.17 ± 0.11		
		Chrys, Calidria	2.8 ± 3.0	0.05 ± 0.04		
		Chrys, NIEHS	4.2 ± 1.2	0.05 ± 0.05		
		Amosite	2.4 ± 1.8	0.31 ± 0.20		
		Crocidolite	2.1 ± 3.6	0.19 ± 0.12		
		Attapulgite	0.8 ± 0.5	0.04 ± 0.03		

*Abbreviations: dG=deoxyguanosine; f=fibers; HTE=hamster tracheal epithelial; OH–dG=hydroxydeoxy-guanosine; mRNA=messenger RNA; MMVF=man-made vitreous fiber; NIEHS=National Institute for Environmental Health Sciences; ODC=ornithine decarboxylase; PBS=phosphate-buffered saline; RCFs=refractory ceramic fibers; RPM=rat pleural mesothelial; UICC=Union Internationale Contre le Cancer.