Annals of Occupational Hygiene Advance Access published January 24, 2005

Ann. occup. Hyg., pp. 1–13

Published by Oxford University Press
doi:10.1093/annhyg/meh098

Survey of the Biological Effects of Refractory Ceramic Fibres: Overload and Its Possible Consequences

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Received 2 July 2004; in final form 5 October 2004

This paper summarizes the biological effects of refractory ceramic fibres (RCFs). RCFs are aluminosilicate glass insulation wools with similar chemical properties to other synthetic vitreous fibres (SVFs) or 'man-made vitreous fibres' (MMVFs). There is concern that RCFs could be significantly more pathogenic than other SVFs. This paper critically reviews the data on which this perception is based. Morbidity studies on workers in RCF manufacturing indicated that, in the United states, RCF exposure was associated with an increased incidence of pleural plaques and in both the united states and Europe with statistically significant changes in some measures of lung function (though not at present exposure levels). No interstitial fibrosis was found. An ongoing mortality study of limited statistical power has failed to indicate any increased incidence of lung cancer or mesothelioma. Findings in several early animal studies led to a large series of inhalation studies where rats exposed to high levels of RCF developed fibrosis and tumours but not those exposed to other SVFs. Similarly hamsters exposed to one sample (RCF1) developed mesothelioma. Subsequent analyses of the data indicated that the RCF used in these experiments had a significantly greater proportion of non-fibrous particles than those present in the other types of SVFs tested or in workplace air. Short-term studies indicated that pulmonary overload occurred at the same as RCF tissue burdens as those in the long-term animal bioassay. When RCFs were prepared in the same way as the other SVFs, a sample resulted with a more representative ratio of particles to fibres; this sample did not produce overload in short-term tests. SVFs have various abilities to persist in the lung tissue and thus accumulate to varying degrees. It is suggested that biopersistence is a key property. While RCFs are among the more persistent they are similar to many other fibre types. The scientific and regulatory implications of these findings are examined.

Keywords: classification; epidemiology; refractory ceramic fibre; risk analysis; toxicology

INTRODUCTION

The chemical and morphological similarities between their products and asbestos has long encouraged man-made vitreous fibre (MMVF) manufacturers to investigate any possible health effects of exposure to their products. For example, as early as the 1940s the Thermal Insulation Manufacturers Association (TIMA) included 'encouraging environmental and occupational health and safety programs' as one of its aims (Pelnar, 1988).

This paper addresses the possible health effects of occupational exposure to refractory ceramic fibres (RCFs). These are aluminosilicate glass wools and are defined by the CAS registry number (CASRN) 142844-00-6. RCFs belong to the class of synthetic vitreous fibres (SVFs), which also includes glass, rock and slag wools. The history, chemical and physical properties, production methods, occupational exposure and commercial applications of the RCFs are summarized in several studies (e.g., Maxim *et al*., 1994, 1997, 2000b). Applications for all of these fibre types are described by the International Agency for Research on Cancer (IARC) in two monographs (1988, 2002) although there are major differences in the taxonomy of fibres between the two reviews.

The desire to identify, control and reduce any risk of occupational illness from the manufacture and use of their products led RCF manufacturers to develop a

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comprehensive Product Stewardship Programme (PSP). The research component of this programme has included both short and long-term animal studies, development of dosimetry and risk analysis models (e.g. Yu *et al*., 1995, 1996; Moolgavkar *et al*., 1999, 2000), development of less biopersistent high temperature fibre wools, industrial hygiene studies of occupational exposure (e.g. Maxim *et al*., 1994, 1997, 1998a,b, 2000a,b; Rice *et al*., 1997) and mortality and morbidity studies of worker cohorts in Europe (Trethowan *et al*., 1995; Cowie *et al*., 2001) and the United States (Lockey *et al*., 1996, 2002; LeMasters *et al*., 1994, 2003; Walker *et al*., 2002).

EPIDEMIOLOGICAL STUDIES

The morbidity of RCF-exposed workers has been monitored in both Europe and in the United States. In a group of workers followed in the United States since 1987 (LeMasters *et al*., 1998) cross-sectional spirometric data demonstrated that 10 years of employment in RCF production was associated with a statistically significant reduction in the forced vital capacity (FVC) for current (165 ml) and past (156 ml) male smokers, respectively. There was no statistically significant decline in FVC among workers who never smoked. For forced expiratory volume in one second (FEV_1) the 10 year decrement (135 ml) was significant only for current smokers. A longitudinal analysis of workers who provided five or more lung function tests between 1987 and 1994 demonstrated no further decline in either FVC or FEV_1 between the initial and last tests in male workers (Lockey *et al*., 1998). Thus, there is no convincing evidence that current occupational exposure levels have resulted in lung function changes. Similar results of an effect on lung function only in smokers or ex-smokers were seen in two cross-sectional studies of RCF workers in Europe (Trethowan *et al*., 1995; Cowie *et al*., 2001). Hansen *et al*. (1999, 2002) reported a similar smoker-only effect on airway obstruction in a population of Danish rock wool production workers. Although the percentage of workers with an airflow obstruction appeared to increase with pack years (see Hansen *et al*., 1999, fig. 2), the effect was statistically significant only for those in the stratum with >40 pack years. There was no X-ray study of the workers and thus no pleural plaques could be reported in the rock wool study.

A radiographic survey of RCF manufacturing workers in the United States included 625 current workers at five manufacturing sites and 383 former workers at two of the five sites. Pleural changes were seen in 27 workers (2.7%). Among employees with >20 years of latency their from first RCF production job or 20 years of duration in a production job, 16 (8.0%) and 5 workers (8.1%), demonstrated pleural changes, respectively. The incidence of irregular opacities at profusion categories >1/0 was similar to other dust-exposed worker populations (Lockey *et al*., 2002).

A European study of RCF workers could find no clear association between pleural plaques and RCF exposure (Cowie *et al*., 2001). To date, chest X-ray studies have not demonstrated any RCF- related increase in interstitial fibrosis. Neither chest X-rays nor clinical observations have shown any excess incidence of lung cancer or any mesotheliomas.

LeMasters *et al*. (2003) examined the mortality of RCF workers. There was no excess mortality related to all deaths, all cancers or diseases of the respiratory system, including mesothelioma (standard mortality ratios [SMRs] for each of these causes were <100). An earlier analysis of these mortality data (Walker *et al*., 2002) designed to explore their statistical power indicated that the experience of lung cancer mortality in the RCF cohort was statistically incompatible with the hypothesis that RCF was as potent as amphibole asbestos (assuming identical cumulative exposure for the cohort). As cumulative exposures and the number of workers were low, the authors could not reject the hypothesis that RCF is as potent as chrysotile asbestos. This mortality study is ongoing.

Notwithstanding limitations, the available epidemiological data support the view that risks from RCF manufacture and use are low. Nonetheless, based on the animal data, several agencies have devised regulations that distinguish between RCFs and other fibre types. In this paper we briefly describe the studies that led to the view that RCF is more hazardous than other SVFs and also summarize more recent work suggesting that among the vitreous fibres there is a continuum of activity and that, with the benefit of hindsight, the opinions expressed on RCFs may have been unnecessarily alarmist and distorted both research and regulation.

Exposures to RCF: concentrations and fibre dimensions

The manufacture and use of RCF wools can liberate fibres and fragments of varying diameter and length. The mean length and diameter of airborne RCF fibres in occupational samples was 20.6 and 1.05 μm, respectively (Mast *et al*., 2000a; Maxim *et al*., 2000b).

Occupational exposures to RCF have been measured and characterized as part of highly structured monitoring programmes (Maxim *et al*., 1994, 1997, 1998a,b, 2000b). Weighted average fibre concentrations have decreased since 1990. The median time-weighted average (TWA) RCF concentration is now $\sim$0.2 fibres ml^{-1} and $\sim$91% of the measured TWAs are beneath 1 fibre ml^{-1}.

Animal studies with RCF

Shortly after commercialization, an animal inhalation experiment was conducted to evaluate the potential toxicity of RCF. The investigators concluded that RCF behaved as an inert dust (Gross *et al*., 1956). As with most other mineral fibres, the injection of RCF into the body cavities of rats was later shown to produce mesothelioma (Wagner *et al*., 1973; Pott *et al*., 1989).

Two further inhalation experiments were carried out during the 1980s. Davis *et al*. (1984) reported that a preparation of RCF produced pulmonary tumours and one peritoneal mesothelioma after long-term inhalation in rats. A similar study by Smith *et al*. (1987) reported that one pulmonary mesothelioma developed in an exposed hamster.

In an attempt to resolve the uncertainties arising from these studies the industry sponsored a comprehensive set of chronic inhalation studies: these were conducted at the Research and Consulting Company (RCC) then located in Geneva.

It was already known that the activity of SVFs in animal experiments depended on three variables: the dose, the fibre dimensions and the fibre biopersistence. Dose can be chosen, as can the sizes of fibres tested, but both can only be controlled with difficulty. Therefore, the first challenge was to prepare test materials suitable for rodent inhalation that also resembled those found in the workplace. Fibres that were too short would be inactive, whereas fibres that were too thick would not be rat-respirable. Previously standard asbestos samples [the UICC (Union Internationale Contre le Cancer) samples] had been prepared solely to be rat-respirable but the techniques used resulted in preparations that were too short for optimum activity. One of the reviewers of this manuscript noted that it is no simple challenge to prepare test materials for bioassays that are representative of those found in the workplace and also appropriately sized for respirability and potential toxicity. Thus, for example, long fibres with diameters >1 μm (and therefore not rat-respirable) are found in the workplace and may be toxic to humans. The UICC asbestos is too short to exhibit significant biological activity, but may be representative of that found in the workplace. And the crocidolite used by McConnell *et al*. (1994) contained unusual amounts of fibres >20 μm long (Walton, 1982; Rödelsperger and Woitowitz, 1993, 1995).

Manufactured and used fibre wools are not suitable for experimental use without substantial modification (Mast *et al*., 1995a,b; Brown, 2000; Brown *et al*., 2000; Bellmann *et al*., 2001). Moreover, the fibrous dust released in normal handling and use cannot be collected in sufficient quantity for an animal bioassay. Scientists at the Carborundum Company (now Unifrax) prepared RCF test samples from the bulk wool by milling, grinding and two stages of water-based separation. When aerosols of these separated samples were generated, they had a fibre size distribution similar to that found in the workplace.

RCF can be produced from calcined kaolin or a mixture of alumina and silica. In Europe, it is manufactured from alumina and silica, and in the United States from Kaolin. Using (what was then) a novel aerosol generation and exposure system, groups of rats and hamsters were exposed at 30 mg m^{-3} to a kaolin RCF dust (RCF1). Rats were also exposed to an equal gravimetric concentration of a silica and alumina fibre (RCF3), a similar fibre containing 15% zirconia (RCF2) and to a simulated 'after-use' fibre (RCF4) made by heating RCF1. In a second study the RCF1 sample was also used in rats at three additional exposure concentrations (the multi-dose study).

The results of these experiments have been extensively reported (see e.g. Bunn *et al*., 1993; Hesterberg *et al*., 1993, 1995, 1996, 1998; McConnell *et al*., 1994, 1995, 1999; Mast *et al*., 1995a,b, 2000a,b; Hesterberg and Hart, 2001). Briefly, only for the highest exposure (30 mg m^{-3}) was the incidence of lung tumours significantly higher than that in either the concurrent control animals or in historical controls for the same rat strain (Rossiter and Chase, 1995). Excluding the highest exposure group, there was no significant dose–response relationship ($P = 0.24$ by the Cochran–Armitage trend test, see Turim and Brown, 2003). Several mesotheliomas occurred in the rats and also in ~40% of the hamsters. These mesothelial tumours were mostly small and did not reduce the average life span of the animals. In both species fibrosis occurred in a way related both to time and concentration.

The tumour incidence for the RCF1 groups of rats is illustrated in Table 1.

In other experiments, glass, slag and rock wools were tested in rats at the same laboratory. For all these experiments it was assumed that 30 mg m^{-3} was the maximum tolerated dose (MTD). Later studies and analysis suggests that the MTD may have been exceeded for some other SVFs (see below).

Rats exposed to similar concentrations of rock, slag and glass fibre showed fewer effects than those exposed to RCF; it is this difference that has served as the basis for several decisions influencing the regulatory status of various SVFs.

As noted above, earlier inhalation studies with RCF were negative or equivocal. Conversely, all the common fibre types are carcinogenic when injected into body cavities although there are significant differences in the dose–response relationships of the different fibre types. Therefore, only the RCC studies suggested that RCFs are qualitatively different from the other SVFs. Additional studies have been

Table 1. Results of the RCC F344 rat inhalation experiments with RCF1

Exposure (mg m^{-3})	Total fibres ml^{-1}	WHO fibres ml^{-1}	Number of animals at risk[a]	Number of tumours				
				Adenomas	Carcinomas	Adenomas and carcinomas	Mesotheliomas	All tumors
0	0	0	256	3	0	3	0	3
3	36	26	123	2	0	2	0	2
9	91	75	127	4	1	5	1	6
16	162	120	124	1	1	2	0	2
30	234	187	121	8	8	16	2	18

[a]Data taken from Yu and Oberdörster (2000). Minor differences exist between the data and assumptions used in these references.

undertaken to understand and interpret these findings and the apparent contradictions between the results from different routes and different fibres.

Were the test materials similar to one another and to workplace exposures?

When the first results of the glass fibre inhalation studies were published it was remarked (Hesterberg *et al*., 1993) that, for the same gravimetric concentration, the RCF aerosols contained fewer fibres than other SVFs tested. This was because the RCF aerosols contained nearly 10 times as much particulate (non-fibrous) material. Later, workplace samples were examined using transmission electron microscopy (TEM) and elemental analysis and it was found that most non-fibrous particles in workplace air, but not the experimental samples, were not derived from RCFs, rather they were of unknown origin (Mast *et al*., 2000a) and presumably identical to those in ambient air. On average, the particle-to-fibre ratios in the experimental RCF1 and in workplace samples differed by a factor of ~18 (Maxim *et al*., 1997; Mast *et al*., 2000a,b). These data show that the RCF tested in the RCC experiments was not representative of that occurring in normal handling and use and was also not similar to the rock, slag and glass fibres tested at RCC.

The significance of particles in the RCF test materials

If the particles made a significant contribution to the RCF animal results and were also found in workplace air samples, it would be appropriate to use such effects in hazard identification or risk assessment. But, as noted above, there is a relatively low proportion of particles in workplace RCF samples. Thus, the presence of these particles in the RCC RCF experiments cannot be ignored.

The low level of particles in the glass, rock and slag wool test materials and finally in the RCF sample (RCF1a), made by the different proprietary process in a different laboratory (Bellmann *et al*., 2001), strongly suggest that the differences in particle content were an artefact of the sample preparation techniques used to prepare the RCFs and MMVFs for use at RCC.

Ultrafine particles of any type can be inflammatory in their own right but the particles in the RCF samples are much coarser than these and so could only have caused disease if they were chemically toxic or present at large enough concentrations to cause pulmonary overload. Simply stated, pulmonary overload is a condition where the bulk of any poorly soluble particulate material in the lung overwhelms alveolar macrophage (AM) mediated clearance. Overload increases with increasing lung dust concentration and ultimately results in, *inter alia*, a persistent inflammatory response, fibrosis and tumors (Morrow, 1994). The mechanism(s) by which lung tumours result from overload caused by fibres and particles are not completely understood (see Morrow, 1994; Oberdörster, 1995; Nikula, 2000; Mossman, 2000 for a discussion).

Overload is well documented in rats. However, it also occurs in other laboratory animals, including hamsters (Muhle *et al*., 1990; Nikula, 2000). Because the effects of overload are seen only at high dust levels, in the absence of a toxic mechanism this condition is not considered relevant for occupational exposures where dust concentrations are very much lower than those used in laboratory bioassays.

OVERLOAD AND ISSUES FOR EXPERIMENTAL ANALYSIS AND DESIGN

For chronic studies of most toxins the MTD may be assessed by monitoring some measure of generalized systemic toxicity, such as weight gain. With chronic inhalation studies of particulate material there is a further complication in that it is now well recognized that excessive particulate exposures cause non-specific effects on the lung in the absence of systemic effects. The lowest lung burden just blocking macrophage mediated lung clearance must be regarded as the MTD for inhalation exposure. This can seriously confound toxicological assessment and risk analysis (Morrow, 1994, 1988; Oberdörster, 1994; Mast *et al*., 2000b).

Thus, for inhalation experiments it is necessary to select some exposures which result in 'high',

dose levels but not so high as to produce overload (Oberdörster, 1997). Failure to select a dose that is sufficiently high might result in a false negative (USEPA, 2004), but selection of an excessively high dose that exceeds the MTD would always cause an effect and thus result in a false positive. In practice, avoiding overload while maximizing the predictive power of an experiment is difficult. The MTD cannot be known in advance and may be impossible to determine from the results of short-term inhalation exposures where a persistent material may not accumulate sufficiently to reach the MTD. The lung dose of persistent particles may continue to increase so that pulmonary overload can develop later. Perhaps the best method of detecting overload is to monitor the thoracic clearance of radioactive tracer particles during the actual long-term study.

Pulmonary overload and fibre clearance

Overload is characterized by the reduction of AM-mediated clearance and its effects on particle lung burdens; however, the effect of overload on fibre accumulation and clearance is more complicated with different fibre size classes clearing differently. Indeed some of the effects of fibres may be due to their being too long for complete phagocytosis and clearance by macrophages. Mathematical modelling by Tran *et al*. (1996, 2003) using data from the RCC studies of glass and rock wool suggested that short fibres and particles clear by the same mechanisms whereas long fibres clear differently. These authors also showed that although the lung burdens of glass fibres was lower than those of RCFs, there was evidence of overload even in the RCC studies with glass fibres.

Bellmann and Muhle (1999) showed that long (>20 μm) RCF1 fibres continued to disappear from the lung even where macrophage clearance, as measured by monitoring the thoracic clearance of radioactive beads, was blocked. Yu *et al*. (1996) found that macrophage clearance of RCF in the RCC studies reduced with both increasing fibre length and increasing lung burden.

All of these clearance studies show that long fibres are not cleared by macrophages, and that they do not dissolve completely but fragment into short fibres rather than into non-fibrous debris. This means that any study that concentrates exclusively on the clearance of longer fibres may not detect overload of macrophage clearance which affects principally (or even only) the clearance of the shortest fibres and non-fibrous particles.

Although the study of mixed-length fibre retention will dilute any effect of reduced macrophage clearance, several analyses of RCF burdens in the RCC experiments indicate that overload probably occurred (Yu *et al*., 1994, 1995, 1996; Yu and Oberdörster, 2000; Mast *et al*., 2000a,b). Figure 1, for example, shows the relationship between the kinetic constant for AM-mediated clearance and the total particulate volume for spheres (open circles) together with values predicted by empirical model (the dashed line) fitted by Yu *et al*. (1996). Although the same

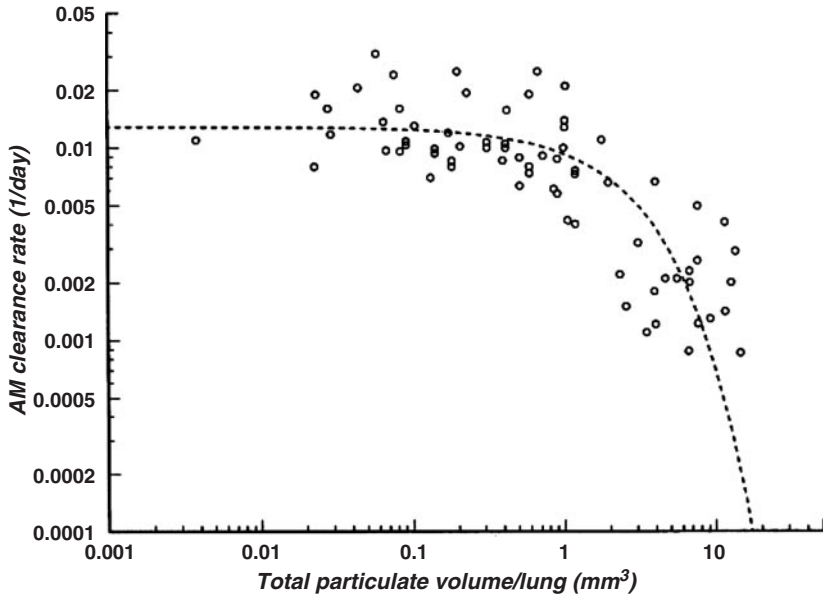

Fig. 1. AM clearance rate (1/day) as a function of the total particulate volume in the rat lung for spherical particles (open spheres). A fitted empirical model is also shown (dashed line). Although the same range of results is not available for RCFs, the data are consistent with the assumption that the fibres behave like spheres of somewhat greater volume (i.e. the fitted curve is shifted below and to the left of the dashed curve shown in this figure). After Yu *et al*., 1996.

range of results is not available for RCFs, the data are consistent with the assumption that the fibres behave like spheres of somewhat greater volume. That is, the fitted curve for fibres is shifted downward and to the left of that shown in Figure 1. [An earlier version of this analysis with lung burden expressed in terms of mass (mg) is found in Yu *et al*. (1994).] The empirical model shown in Figure 1 is of the form,

$$\lambda_m = \lambda_{mo}\exp\left[-a(V/V_{AM})^b\right] \qquad (1)$$

where λ_m is the kinetic constant for AM-mediated clearance (a function of V), λ_{mo} is the kinetic constant for AM-mediated clearance at low lung burden, V is the total volume of RCF retained in the lung at time t, V_{AM} is the total volume of AMs in rats (26 mm^3), and a and b are constants estimated by statistical methods.

For non-fibrous particles (shown in Fig. 1) the parameters are estimated to be $\lambda_{mo} = 0.013\ day^{-1}$, $a = 7.2$, and $b = 0.95$; the corresponding estimated values for RCF are $\lambda_{mo} = 0.0058\ day^{-1}$, $a = 17.4$, and $b = 0.95$. Overload occurs at and beyond a value V where the kinetic constant is lowered from the value λ_{mo}. This AM submodel was incorporated into an overall RCF dosimetry model by Yu *et al*. (1996), which also included breakage and dissolution removal mechanisms for fibres.

Did the particle contamination contribute to the effects of exposure to RCFs?

There are three ways in which the high particle concentrations in RCC RCF samples could contribute to the effects of exposure.

- Particles (as well as fibres) will contribute to overload and reduce the rate of AM-mediated clearance, resulting in the accumulation of higher lung burdens of (at least short) fibres and particles.
- Sufficiently high concentrations of even innocuous dusts (e.g. toner, talc, TiO_2) can cause inflammation and lung tumours in rats (see e.g., Heinrich, 1994; Hext, 1994; Morrow, 1994, 1988; Oberdörster, 1994; ILSI Risk Science Institute Workshop Participants, 2000; Nikula, 2000; Yu and Oberdörster, 2000). As noted by Morrow (1994):

  Dust overload is a pulmonary condition induced by excessive amounts of relatively benign, insoluble dusts in the lungs of rats (and a few other species) characterized initially by increased dust retention. The condition of overload increases with increasing lung dust content and progressively determines the pathophysiologic state reached which ultimately includes a persistent inflammatory response, irreversibly suppressed dust clearance, increased interstitial dust uptake, and the emergence of adverse effects, e.g. fibrosis and tumors.

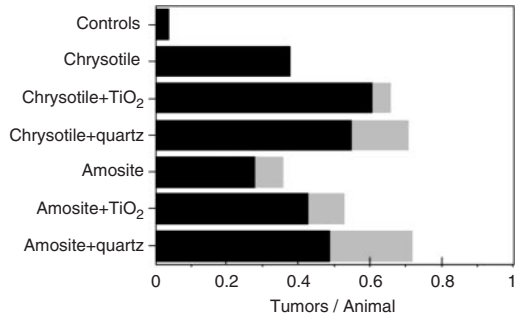

Fig. 2. Number of tumours (lung or mesothelioma)/animal resulting from mixed fibre/dust exposure in a 1 year rat inhalation study (Davis *et al*., 1991). Increase in tumour incidence resulted when rats were co-exposed to particulate (either innocuous or pathogenic) matter and fibre (Pearson chi-square 50.67, $P < 0.001$; likelihood ratio chi-square 59.34, $P < 0.001$).

- Co-exposure of particles and fibres can result in synergistic effects in causing lung tumours or mesotheliomas (Davis *et al*., 1991; Davis, 1996). For example, Fig. 2 shows the number of tumours (lung and mesothelioma) in a 3 year rat inhalation study (1 year exposure with a 2 year follow-up) conducted by Davis *et al*. (1991). The incidence of tumours increased from co-exposure to even benign dust.

The practical significance of excessive particle concentrations in a fibre bioassay is that these confound the interpretation of the experimental results. In the absence of other data, it is not possible to determine the extent to which the observed experimental outcome is due to the fibres, the particles or to some combination of both. This confounding complicates decisions regarding carcinogen classification and quantitative estimation of risks.

When it was recognized that the original RCF samples were not representative of workplace aerosols, a new RCF sample, termed *RCF1a*, was prepared by the Manville Corporation using the techniques previously employed to prepare the glass, rock and slag wool samples (Brown *et al*., 2000; Bellmann *et al*., 2001). RCF1a was prepared from the same starting material as RCF1 and was thus chemically identical. It had a similar fibre size distribution but contained far fewer non-fibrous particles. Thus the particles in the original RCF samples arose as a function of the preparation technique and not due to some property of the material itself. Unlike RCF1, RCF1a had a similar particle-to-fibre ratio to both workplace air and to the glass, rock and slag wool samples used in the RCC studies (Bellmann *et al*., 2001).

Samples of the original, particle-rich, RCF preparations had been provided to many laboratories. One such sample of RCF1 was used in a short-term inhalation study (Creutzenberg *et al*., 1997). Female Wistar rats were exposed for 6 h per day, 5 days per week, for 3 weeks. After various periods the lung dust burdens

and several measures of biological response were measured. Four days after the end of the 3-week exposure, a subset of the animals was briefly exposed to an aerosol of radioactive scandium oxide; the thoracic radioactivity of the tracer-exposed animals was measured once or twice weekly for up to 90 days. The clearance half-time for the scandium oxide particles, determined from the measurements of thoracic radioactivity, provides a measure of the rate of macrophage clearance. The RCF1a sample was used in a repeat of this study that was designed so that the exposure concentrations of fibres longer than 20 μm in the two studies were as similar as possible.

A comparison of these studies determined, *inter alia*, that the clearance kinetics of the long (>20 μm) fibres was similar for both samples; for RCF1a the half-time was 62 days (57–67 days, 95% confidence interval) compared with 77 days (71–84 days) for RCF1. However, the clearance half-times of WHO fibres and of all fibres (those particles with an aspect ratio of more than 3) were twice as long after RCF1 exposure. For example, the clearance half-time for all fibres after exposure to RCF1 was 102 days, compared with 58 days for RCF1a (Bellmann *et al*., 2001). There was an even greater effect on tracer particle clearance with the clearance of $^{46}Sc_2O_3$ particles (Table 2) increasing from 60 days in control rats to 80 days after RCF1a exposure but to 1200 days after exposure to RCF1.

Thus the doses of RCF1, which caused the collapse of the macrophage clearance of particles, had a much smaller effect on long fibre clearance. The lung burdens in these experiments are presented in Table 3 at 3 days after exposure ceased and after a further 12 months. Exposures to similar numbers of fibres resulted in similar lung fibre burdens but caused pulmonary overload, as detected by the collapse of particle clearance, with RCF1 but not RCF1a. Thus, this overload must be either a response to the lung burden of non-fibrous particles or to the combined burden of particles and fibres; it cannot be due to any effect of the fibres alone.

Table 2. The half-times for the clearance of radioactive scandium oxide particles from the thorax of rats after short inhalation exposure

Group	Half-time for alveolar tracer clearance (95% confidence limits) (days)
Control	60 (49–77)
RCF1a	80 (71–91)
Earlier study (Creutzenberg *et al*., 1997)	
Control	66 (58–88)
RCF1	1200 (573–∞)

Full details in Bellmann *et al*. (2001).

The biological effects of short-term exposure to RCF1 were both qualitatively and quantitatively greater than those resulting from exposure to RCF1a (Brown *et al*., 2000; Bellmann *et al*., 2001). These inflammatory conditions are similar to those that result from overload lung burdens of other materials including 'inert' dusts. This could be simply due to the pulmonary overload detected by the effects on tracer clearance.

While differences in lung fibre burdens between RCF1 and RCF1a were small, there were significant differences between those fibres found in the lymph nodes (Table 4). With the particle contaminated RCF1, more than 10 times the number of short fibres (median length ∼4 μm) were translocated to the lymph nodes and continued to accumulate there even after exposure ceased.

Mast *et al*. (2000b) compared the lung burdens of RCF1 in the carcinogenicity studies at RCC with those shown to cause overload in the 3 week study. They concluded that:

> This level of RCF1 fibre in the lung [that which caused the collapse of pulmonary clearance] was attained in the RCC studies in approximately 17, 24, and 40 wk at the exposure levels of 30, 16, and 9 mg m^{-3}, respectively. [Parenthesis and contents added].

It is therefore a reasonable assumption that lung burdens in the RCC experiments with RCF1 were at overload concentrations for most of the animals' lifespan. With the possible exception of mesotheliomas this could account for all the pathology observed in the absence of any other mechanism.

The few mesotheliomas in the rat, and the many in the hamster, might be due to the synergism between

Table 3. The burden of particles and fibres in the lungs of rats after 3 week exposures (Bellmann *et al*., 2001)

Treatment/time after exposure ceased	Numerical burden (×10^6/lung)			Mass concentration (mg/lung)	
	Fibres L > 20 μm	WHO fibres	Particles	Fibres	Particles
RCF1a					
3 Days	4.999	25.51	6.9	0.463	0.045
12 months	0.074	1.15	0.6	0.012	0.002
RCF1					
3 Days	3.279	29.60	102.4	0.666	1.181
12 months	0.119	2.28	2.1	0.028	0.027

Table 4. The fibre burdens in the lung associated lymph nodes [experiments described in Bellmann *et al*. (2001)]

Time after exposure	Lung associated lymph node fibre burden 10^6/animal (SD)			
	RCF1		RCF1a	
	All fibres	WHO fibres	All fibres	WHO fibres
3 days	0.109 (0.069)	0.032 (0.019)	0.008 (0.002)	0.003 (0.000)
1 month	0.185 (0.032)	0.051 (0.024)	0.018 (0.002)	0.007 (0.001)
3 months	0.808	0.209	0.024 (0.006)	0.009 (0.005)
12 months	0.955 (0.682)	0.306 (0.197)	0.038 (0.021)	0.017 (0.041)

No fibres longer than 20 μm were found in the lymph nodes.

fibres and particles described in the work of Davis and his colleagues (Davis *et al*., 1991; Davis, 1996). Interpretation of the RCC hamster results is difficult because multiple doses were not included and the hamster might not be a suitable model for the study of mesothelioma. Warheit and Hartsky (1994) questioned the suitability of the hamster model for assessments of any risk of mesothelioma in humans and concluded, 'rats appear to be a sensitive model for assessing lung tumorigenic and mesotheliomagenic responses in humans'.

To examine further the effects of RCF a 90 day subchronic study was undertaken which included a sample of high purity RCF and a non-fibrous particulate with the same chemical composition (Bellmann *et al*., 2002; Brown *et al*., 2002). Unfortunately, the fibre sample contained ~30%, by weight, of non-fibrous material. Although the particles were larger than those in RCF1, so that the ratio of fibres to particles was nearer to 1:1, however the RCF sample was more similar to RCF1 than RCF1a. Exposure to the non-fibrous particulate had more effect on most endpoints than exposure to the fibre. As with RCF1, exposure to the particles caused the collapse of macrophage-mediated clearance, as measured with radioactive test particles ($T_{1/2}$ > 500 days where control ≈ 60 days). The effect of RCF exposure still caused a significant reduction in clearance ($T_{1/2} \approx 400$ days) while a sample of amosite asbestos tested at the same time produced only slight retardation of clearance ($T_{1/2} \approx 100$ days). The inflammatory effects of the exposures were proportionate to the degree of retardation of tracer clearance (overload) and only secondarily to fibre type. In contrast cell proliferation, in both terminal airways and lung parenchyma, was proportional to fibre burden rather than general inflammation. Proliferation was most affected by amosite and for this endpoint alone RCF produced more effect than the non-fibrous particles. The effects of amosite persisted for the entire year of follow-up, where as that for both RCF and particles recovered earlier.

How might overload affect mesothelioma?

If the normal macrophage clearance to the gut is blocked, then higher doses of fibre could reach the pleura. Mesothelioma could result from this redistribution of fibre clearance leading to higher doses in that tissue. Increased pleural and subpleural fibre accumulation would cause more inflammation and perhaps increased deposition onto the parietal pleura (see Zocchi, 2002) where, in humans, this results in the localized accumulation of dust as 'black spots' (see e.g. Mitchev *et al*., 2002). It may be that fibres do not even need to enter the pleural cavity as it has been suggested that subpleural inflammation can lead to mesothelial proliferation (Davis, 1989). In the rat, inhaled fibrous dust has been shown to localize in the periphery of the lungs under the pleura (Morgan *et al*., 1977).

RISK ANALYSIS RESULTS

The main reasons for animal testing are to use the results to identify any hazard and to predict risk to humans. In the absence of epidemiological evidence, a valid and accurate dose–response animal study is needed. Many injection studies have provided dose–response data, but it is unclear how to use doses injected into the body cavity of rats to derive equivalent human doses and/or exposures.

For this reason inhalation experiments are necessary and the incidence of lung cancers in the RCC rat studies with RCF might be used for this purpose. However, in addition to the usual challenges associated with species and dose extrapolation, the RCF data are confounded with the presence of particles with an unknown contribution to toxicity. Despite these limitations there have been several attempts to estimate risks to RCF-exposed human cohorts based on some, or all, of the RCC data (see e.g. Moolgavkar *et al*., 1999, 2000; Yu and Oberdörster, 2000; Maxim *et al*., 2003; Turim and Brown, 2003). The most sophisticated analyses incorporate both a dosimetry and a potency model. The specific risk estimates associated with occupational exposure throughout a working lifetime vary depending upon the dosimetry model parameters, the basis for normalization of lung burdens (between rats and humans) and other factors. However, the maximum likelihood estimates (MLE) of the incremental lung cancer risk associated with working lifetime exposure to RCF at the high end of the levels typically found in the workplace were $< 1 \times 10^{-3}$. These estimates do not include any allowance for the possibility that

the observed biological effects in rats were affected by pulmonary overload and thus by the presence of particles.

These risk analyses are primarily based upon the data set reproduced in Table 1. Moolgavkar *et al*. (2000) extended earlier calculations (Moolgavkar *et al*., 1999) by using many of the same assumptions but analysing the hypothesis that all fibres present in the lung have equal carcinogenic potency, whatever their composition. This has been summarized as 'a fibre is a fibre'. This hypothesis assumes that any cancer risk for SVFs is determined entirely by the lung fibre burden and its temporal profile both during and after exposure. Many SVFs have similar specific densities and the airborne fibres have similar size distributions and, therefore, their pulmonary deposition patterns would be similar. In this case only the fibre's biopersistence contributes to any differences in lung burden and ultimately tumour outcome. For several SVFs Moolgavkar *et al*. constructed age-dependent profiles for the lung burdens of various sized fibres, the two-stage clonal expansion model incorporating initiation and promotion or both was used to describe potency. This analysis fitted dose–response models to each of the SVFs tested at RCC separately and in various combinations of fibre types. The likelihood ratio procedure was used to test whether the model with separate parameters for each of the fibre types fitted the data better than the assumption of common parameters for all fibre types.

This analysis indicated that the RCC data for all SVFs were indeed consistent with the sole determinant of lung cancer risk being the time-dependent lung burden. However, the responses at the highest exposure level of RCF (30 mg m^{-3}) were more than those that could be predicted from lung fibre burden alone. These high dose responses were statistical outliers suggesting that another mechanism had contributed to the tumour incidence in the groups exposed to 30 mg m^{-3} of RCF1 and RCF3. When these two groups were deleted from the data, the likelihood ratio tests indicated that the tumour response in both the remaining RCF groups and those from other SVFs was homogeneous. That is differences in tumour response could be related to the exposure concentrations of different fibre types using only one fibre property, biopersistence, and its effects on lung burdens.

One interesting conclusion of this work is that the traditional positive/negative dichotomy of bioassay outcomes is too simplistic. According to the Moolgavkar analysis all SVFs have essentially the same oncogenic potential; the reason that some studies fail to show a statistically significant response is that these studies do not have sufficient statistical power to detect the small level of incremental risk associated with a fibre that clears rapidly. Rather than a dichotomy, there is a continuum of biopersistence and therefore a continuum of risk presented by various SVFs. This concept was articulated further in a subsequent study (Moolgavkar *et al*., 2001). The authors concluded that, because the key differences among SVFs were related to biopersistence (or biodurability) rather than any known measure of oncogenic potential, a well designed and calibrated biopersistence study might be sufficient to estimate any risk from an SVF. A full traditional carcinogen bioassay may be unnecessary and fewer animals would be used.

In terms of biopersistence, the weighted half-time of long (>20 μm) RCF1a fibres after short-term inhalation was reported as 55 days (Hesterberg *et al*., 1998; Bernstein *et al*., 2001). This indicates RCF is more biopersistent than some fibres, but is by no means the most biopersistent of the SVFs and is at least an order of magnitude less biopersistent than amphibole asbestos (either amosite or crocidolite). Figure 3 provides a sample of weighted half-times (Hesterberg *et al*., 1998). SVFs with similar half-times to that for RCF1a in this sample include MMVF21 (rock wool), MMVF32 (E glass) and MMVF 33 (475 glass). These weighted half-time estimates were determined using a similar protocol, apply to fibres of similar (>20 μm) length and therefore can be compared.

Moolgavkar *et al*. (2000) concluded that the chemical composition of an SVF is important only insofar as it determines the rate of clearance of fibres. This result is also consistent with the hypothesis that particulate matter was responsible for the apparent outliers at the high dose level. As to specific occupational risks, it was estimated that the working lifetime risk of lung cancer associated with exposure at an average of 1 fibre ml^{-1} to RCF or any fibre of comparable biopersistence would be $\sim 5 \times 10^{-5}$, a much lower

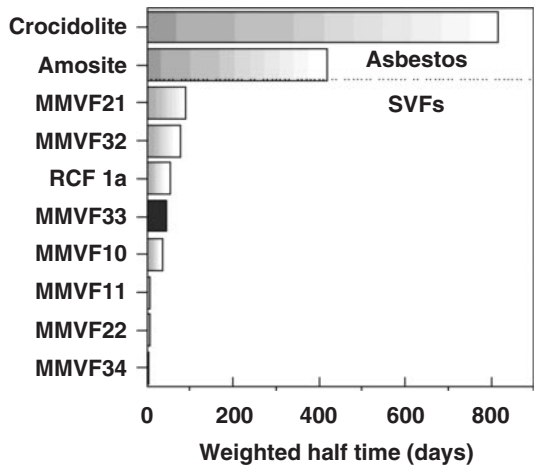

Fig. 3. Weighted half-time of long (>20 μm) fibres for two types of amphibole asbestos and several SVFs after short-term inhalation (Hesterberg *et al*., 1998).

estimate than one derived from the data including the apparent outliers.

These studies have been extended to include other dosimetry models to extrapolate between rats and humans (Turim and Brown, 2003). The whole basis for such extrapolation is further explored in Maxim *et al*. (2003).

Taken together, the more recent work of Mast *et al*. (2000a,b), Brown *et al*. (2000), Bellmann *et al*. (2001), Moolgavkar *et al*. (2000) and the most recent epidemiological data (Walker *et al*., 2002; LeMasters *et al*., 2003) indicate that risks posed by RCF exposure must be lower than estimated from earlier consideration of the RCC results.

RCFs are not consumer products and relatively few people are exposed. The industry has sponsored both morbidity and mortality studies on cohorts occupationally exposed in RCF manufacture and processing; these are briefly reviewed in the introduction above.

RCF AND REGULATION

Agencies charged with carcinogen classification and the regulation of exposures have been apt to use the RCC results at their simplest 'face' value. This has led to a view that RCFs are qualitatively different from other SVFs. In principle, regulations/policies dealing with classification recognize the significance of exceeding the MTD and the occurrence of pulmonary overload as sources of false positive results. For example, in Europe *Annex VI of the General Classification and Labelling Requirements for Dangerous Substances and Preparations* (Directive 67/548/EEC) list the criteria distinguishing between Category 2 (risk phrase 'may cause cancer') and Category 3 (risk phrase 'limited evidence of carcinogenic effect'); these criteria include several that reduce the significance of experimental tumour induction in view of possible human exposure. Included are carcinogenic effects 'found only at very high dose levels exceeding the maximal tolerated dose'. These criteria define two subcategories for Category 3. Subcategory 3(b) is described as:

> substances which are insufficiently investigated. The available data are inadequate, but they raise concern for man. This classification is provisional; further experiments are necessary before a final decision can be made.

In the United States, one of the recommendations of the Presidential/Congressional Commission on Risk Assessment and Risk Management (1997) was that regulatory agencies should distinguish between tumour responses that are predictive and those that are not. One of the examples given of the latter is where there is 'overwhelming of clearing mechanism' in the rat lung. And a recent USEPA (2004) Staff Paper on risk assessment principles and practices notes that 'failure to reach a sufficient dose reduces the sensitivity of the studies', but stresses 'the importance of establishing that the MTD has not been exceeded'. Thus, the finding that overload occurred in the RCC RCF experiments should have regulatory significance on both sides of the Atlantic.

RCF is presently classified as Category 2 by the European Union and in Group 2B (possibly carcinogenic to humans) by IARC in 1988 (IARC, 1988), a decision reaffirmed in 2002 (IARC, 2002). However, in the years since the results of the RCC studies were first published, our knowledge of fibre toxicology generally (and that for RCF in particular) has evolved substantially. The possible effects of co-exposure to particle–fibre mixtures provide a cautionary tale for those engaged in the design of future animal bioassays with other fibres. The works of many investigators (including Moolgavkar *et al*., 2000, 2001) have highlighted the importance of fibre biopersistence and suggested alternative experimental designs for estimating the risks posed by fibres.

In retrospect, the RCF/RCC study should have been designed differently. The positive control used in the study should have been matched in terms of fibre length and, most importantly, a sample preparation method for RCF should have been used that more nearly matched the ratio of particles-to-fibres to that found in workplace samples. For the other samples tested at RCC this was the case. Unfortunately, the different methods of preparation might have contributed as much to the observed results as material differences among the substances tested. Moreover, no one should consider a chronic inhalation study without contemporaneous tests for the integrity of AM-mediated clearance. The particulate content of the RCF1 might well have significantly increased the biological effects observed in the RCC experiments. It would be better to test this by carrying out a chronic inhalation experiment with RCF1a. This would allow a direct assessment of the carcinogenicity of RCF as compared with other fibre types.

In total, the RCC experiments were important and have stimulated a great deal of additional research that increased our understanding of fibre toxicology and pointed the way to improved experimental designs. The RCC experiments are less useful, however, for assessing RCF hazards and risks. Limitations of these studies, discussed at length above, preclude a definitive assessment of the effects of exposure to RCF and should be reflected in classification decisions. In particular, in the European classification system, Category 3(b) seems more appropriate than Category 2 for RCF. The available animal studies, though now voluminous, are inadequate—because the MTD was exceeded, the material tested was not representative of that to which humans are exposed and the contribution of

the particulate matter to overall toxicity cannot be established. In a 2003 guidance document issued to the Working Groups on Classification and Labelling (ECBI/55/03 Add.7) the Directorates-General Environment and Enterprise and the European Chemicals Bureau noted that classification 'recommendations have to be based on solid science and clear evidence and be strictly in line with the provisions and criteria of the Directive'.

To be sure, the RCC results indicate that some concern regarding occupational exposure to RCF remains justified. Though overload and co-exposure to a non-representative amount of particulate matter have confounded the interpretation of the RCC studies—the study results do not exonerate RCF. But, neither do they justify the present EU classification. Pending additional data and analysis, it is certainly prudent to continue the RCF PSP to control and, where possible, reduce exposures.

Acknowledgements—This study was sponsored by ECFIA, a European trade association for the manufacturers of high-temperature insulating fibres including RCF. We wish to acknowledge the many helpful comments of several reviewers. The revisions in response to these comments have considerably improved the paper.

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