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Clearance of Refractory Ceramic Fibers (RCF) from the Rat Lung: Development of a Model

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Chronic exposure and postexposure experiments have been recently performed in rats to evaluate the biological responses of inhaled refractory ceramic fibers (RCF) at different concentration levels. The lung burden data in the accessory lobe of the rat lung were collected during and after different exposure and postexposure periods. The size distribution of retained fibers in the lung at different time points was also measured. We used these data to develop a mathematical model of fiber clearance from the rat lung. It was found that the clearance rate did not depend significantly upon fiber size but there was a clear dependence on lung burden. As lung burden increased, the clearance rate was found to decrease. An empirical equation was derived for the clearance rate as a function of lung burden. At low burdens, rats had a retention half-time of about 126 days for RCF compared to a typical half-time of about 60 days for insoluble nonfibrous particles.

INTRODUCTION

It has been generally recognized that the health effects of inhaled fibers depend not only upon the fiber type but also upon the size and number (mass) of fibers accumulated in the lung. Recently, chronic nose-only exposure experiments in rats have been conducted by the Research and Consulting Company (RCC) in Geneva, Switzerland, to evaluate the biological responses of refractory ceramic fibers (RCF). In an earlier paper (Yu *et al.*, 1994), we presented a mathematical model of RCF deposition in the rat lung. The purpose of this paper is to develop a mathematical model which describes the clearance kinetics of RCF and to determine the clearance rate based upon the experimental data. By combining deposition and clearance modeling results, accumulation of RCF in the rat lung at any time during exposure can be predicted. The ability to describe the accumulation kinetics of RCF in the rat lung is a prerequisite for a subsequent model of the deposition and retention of these fibers in the human lung.

The importance of the rat inhalation studies with RCF is based on the finding of a significant lung tumor rate in the high-exposure groups, i.e., at 30 mg/m^3 (approximately 234 fibers/cm^3) the observed incidence of lung tumors was about 13% whereas lower concentrations did not produce statistically increased numbers of tumors. What is the significance of these findings for human RCF exposure? Should the results seen in the rat studies at high concentrations be extrapolated to humans, and can appropriate models be developed to estimate human risk at relevant low workplace-exposure concentrations? The modeling efforts described in this paper with respect to the clearance kinetics of inhaled RCF in the rat lung are a first step to address this complex issue.

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RCF INHALATION STUDIES

Two separate inhalation studies were conducted by RCC. In the first study, rats were exposed to four different types of presized RCF (kaolin, zirconia, high purity, and "after service") at 30 mg/m^3 (approximately 234 fibers/cm^3). The after-service fiber was produced by heating kaolin fiber in a furnace to 2400°F for 24 hr and the resulting fiber was substantially shorter and thicker than the starting material. The physical dimensions of the different types of RCF are listed in Table 1. The second study was a multidose exposure in which rats were exposed to kaolin RCF at concentrations of 3, 9, and 16 mg/m^3 (approximately 36, 91, and 162 fibers/cm^3). One hundred and forty male Fischer rats at 11 weeks of age were used in each exposure group. The exposure pattern was 5 days/week and 6 hr/day for a total period of 104 weeks. After 13, 26, 39, 52, 65, and 78 weeks of exposure, three to six rats were sacrificed and an additional three to six rats were removed from exposure (recovery) until the end of 104 weeks. At this time point, another three to six rats as well as recovery rats were sacrificed. All remaining animals were sacrificed after an additional period of 23 weeks when approximately 20% survival had been reached.

The lung burden and fiber dimensions were measured in the accessory lobe of the rat lung. After rats were sacrificed, the lobes were immediately deep frozen. The frozen lobes were dehydrated in acetone and dried to constant weight. The dry tissue weight was recorded and the lobe was plasma ashed. The ash from each lung was dispersed in distilled water and aliquots were filtered onto Nuclepore membrane filters for measurements of fiber size and lung burden by scanning electron microscopy. A description of the experimental procedure and original data has been reported elsewhere (Glass *et al.*, 1992; Mast *et al.*, 1993).

CLEARANCE MODEL AND KINETIC EQUATIONS

Previous studies (e.g., Timbrell and Skidmore, 1971; Morgan *et al.*, 1978; Roggli and Brody, 1984) have shown that the clearance rate of asbestos and glass fibers is dependent upon fiber size, and in particular, the fiber length. Short fibers were found to clear from the lung more efficiently than long fibers. In other studies (Bolton *et al.*, 1983; Vincent *et al.*, 1985), it was found that the clearance was slowed at high lung burden. Inhalation experiments performed in rats using amphibole fibers, such as amosite and crocidolite, resulted in a continuous buildup of fibers in the lung when the lung burden exceeded a threshold level. This

TABLE 1
REFRACTORY CERAMIC FIBERS (RCF) USED IN EXPOSURE AND POSTEXPOSURE EXPERIMENTS

Type	$\bar{d}_f$	$\bar{l}_f$	σ_d	σ_l
RCF 1 (kaolin)	0.82	15.9	1.89	2.4
RCF 2 (zirconia)	0.88	12.8	1.92	2.5
RCF 3 (high purity)	0.85	17.4	1.99	2.4
RCF 4 (kaolin after service)	1.22	9.8	1.68	2.0

Note. $\bar{d}_f$, geometric mean diameter; $\bar{l}_f$, geometric mean length; σ_d, fiber diameter geometric standard deviation; σ_l, fiber length geometric standard deviation.

is reminiscent of a particle "overload effect" which had been observed with particles of materials formally characterized by "nuisance dust" (e.g., Ferin and Feldstein, 1978; Chan *et al.*, 1981), and indeed Bolton *et al.* (1983) used the term overload to describe the accumulation kinetics of the amphiboles. When the lung burden of nuisance dust reached a level of 1–3 mg/g of lung in chronic rat inhalation studies, a significant prolongation of particle clearance was found (e.g., Muhle *et al.*, 1990). Morrow (1988) hypothesized that this prolongation of particle clearance is caused by a volumetric overloading of the alveolar macrophages with subsequent loss of their mobility. Whether a potential overload-related prolongation of normal lung clearance also occurs with RCF is not known.

Mathematical models have often been used to describe the clearance kinetics of inhaled particles in the lungs (see, for example, Oberdorster, 1989, for a review). Because of the complexities in the clearance process of fibrous dusts, only a few model studies have been conducted specifically for fibers. Yu *et al.* (1990, 1991) proposed a model for the clearance of chrysotile asbestos fiber from the rat lung, taking into account the size-dependent clearance rate and fiber splitting. Vincent and his colleagues (Bolton *et al.*, 1983; Vincent *et al.*, 1985; Jones *et al.*, 1988) and Yu and Asgharian (1990) developed models to simulate the effect of lung burden on the clearance of amosite fibers. Because only total lung burden data were available for modeling studies, all of these works considered the alveolar region of the rat lung as a single compartment. Thus, the clearance rate evaluated from the model represents the total effective alveolar clearance rate of fibers through different pathways.

The single compartment model will also be used in this study. Let $n(d_f,l_f,t)$ be the number of fibers of diameter d_f and length l_f in the lung at time t. The kinetic equation which describes the time variation of $n(d_f,l_f,t)$ in the lung is then

$$\frac{dn(d_f,l_f,t)}{dt} = r(d_f,l_f,t) - \lambda(d_f,l_f,M)n, \tag{1}$$

where $r(d_f,l_f,t)$ is the deposition rate, and $\lambda(d_f,l_f,M)$ is the clearance rate in which M is the total fiber mass in the lung, given by

$$M = \int_0^\infty \int_0^\infty \rho n(d_f,l_f,t) \left(\frac{\pi}{4} d_f^2\right) l_f dd_f dl_f = Nm, \tag{2}$$

where ρ is the fiber mass density, N is the total number of fibers, and m is the mean fiber mass.

Figures 1a and 1b compare the fiber size distribution of RCF 1 measured in the rat lung after 52 weeks of exposure to a fiber concentration of 3 mg/m^3 with the fiber size distribution found 52 weeks postexposure. These data show that the size distribution does not change significantly during postexposure. The data of the geometrical mean diameter and length of the recovered fibers in the lung before and after recovery for different exposure and postexposure conditions are shown in Fig. 2. Again, no consistent change in fiber size is observed between the different time points and between exposure and postexposure. This surprising

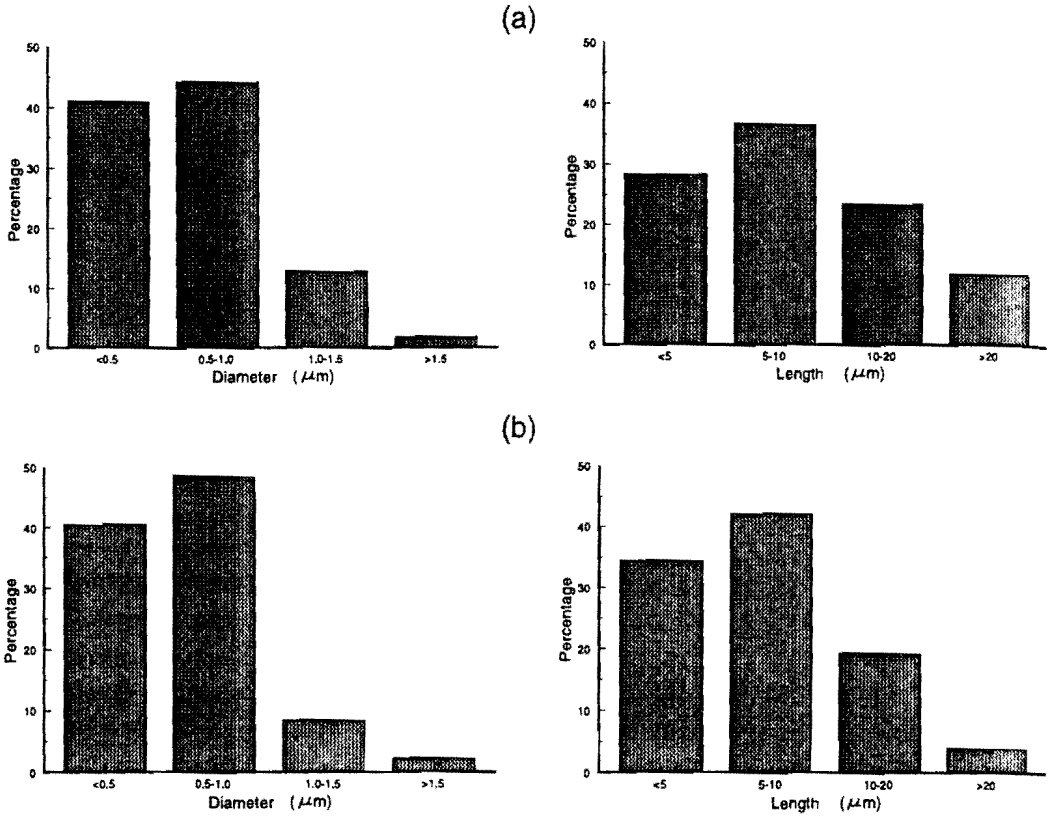

FIG. 1. Size distribution of RCF 1 recovered from the rat lung at the beginning and the end of a postexposure experiment. The experiment was for an exposure period of 52 weeks at a concentration of 3 mg/m^3 followed by a postexposure period of 52 weeks. (a) Beginning of the postexposure and (b) end of the postexposure.

experimental result, similar to that found by Hammad (1984), implies that all RCF are cleared at about the same rate regardless of their size. Although there may be a possibility of breakup of long thin fibers into short ones, the evidence from the data is not strong. Based upon this experimental finding, we assume that $\lambda(d_f,l_f,M)$ in Eq. (1) is independent of d_f and l_f and is only a function of M.

During postexposure, $r = 0$, Eq. (1) is reduced to

$$\frac{dn}{dt} = -\lambda(M)n. \tag{3}$$

Integrating Eq. (3) over all fiber sizes, it becomes

$$\frac{dN}{dt} = -\lambda(M)N, \tag{4}$$

in which $M = Nm$.

DETERMINATION OF CLEARANCE RATE

Equation (4) is used to determine $\lambda(M)$ from the data of different postexposure (recovery) experiments where M is the fiber lung burden at the beginning of a postexposure. The solution of Eq. (4) is

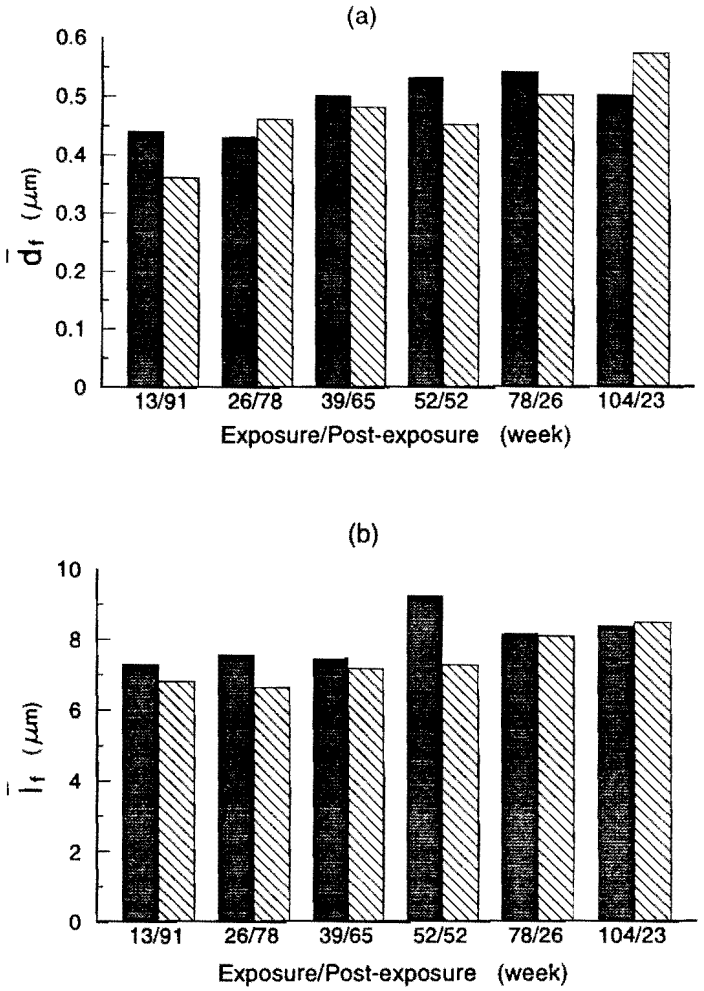

FIG. 2. Variations of the geometrical mean diameter and length of RCF 1 for different exposure and postexposure periods at a concentration of 30 mg/m^3. (a) Diameter and (b) length.

$$\frac{N_t}{N_0} = exp(-\lambda t). \tag{5}$$

At $t = T$, we obtain from Eq. (5) that

$$\lambda(M) = -\frac{1}{T} ln \frac{N_T}{N_0}, \tag{6}$$

where N_0 and N_T are, respectively, the total number of fibers in the lung before and after a recovery period T. Strictly speaking, M in Eq. (1) is a function of t, but the change in M during a recovery period is very small compared to the variation of M caused by different exposure concentrations and time periods in the exposure experiments.

Figure 3 shows the result of λ as a function of M calculated from Eq. (6) for different types of RCF. The data of λ for insoluble nonfibrous particles previously compiled by Yu *et al.* (1989) are also shown. It is seen that λ decreases with M for

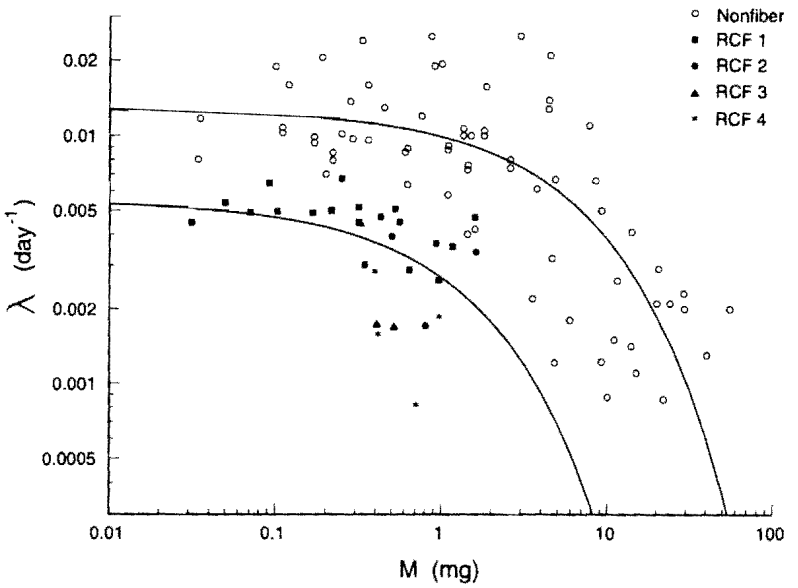

FIG. 3. Clearance rate λ versus M for RCF and nonfibrous insoluble particles. M of RCF does not include nonfibrous particle contribution.

RCF, similar to that observed in nonfibrous particles. However, the value of λ for RCF at a given mass lung burden is considerably lower than that of nonfibrous particles. It is also seen that for the same lung burden M, RCF 4 has a lower clearance rate than others. RCF 4 contains approximately 27% free crystalline silica in the form of crystobalite. The presence of this crystalline silica in RCF 4 may cause an additional reduction of clearance rate due to its high cytotoxicity.

It is possible to fit the data of λ by an empirical equation of the form

$$\lambda(M) = a \exp(-bM^c), \qquad (\text{day}^{-1}) \tag{7}$$

where a, b, and c are constants and M is the lung burden in milligrams. For RCF, we found $a = 0.0055$, $b = 0.72$, and $c = 0.66$ and for nonfibrous particles, $a = 0.0128$, $b = 0.26$, and $c = 0.67$. At small M, Eq. (7) gives $\lambda = 0.0128\ \text{day}^{-1}$ for nonfibrous particles corresponding to a retention half-time of 54 days, and $\lambda = 0.0055\ \text{day}^{-1}$ for RCF, or a half-time of 126 days. Figure 4 shows comparisons of the predicted clearance curves using these values of λ with the data of RCF 1 obtained by RCC and the data of carbon black obtained by Strom *et al.* (1989). Carbon black consists of submicrometer nonfibrous particles; the faster clearance of these particles in comparison with RCF 1 is obvious from Fig. 4.

Bellmann *et al.* (1987) recently made measurements on the persistence of man-made mineral fibers in the rat lungs. The fibers were administered to the lung by intratracheal instillation. For ceramic fibers, they found a clearance rate of 0.0011 day^{-1} at a lung burden of about 2 mg. This is very close to the value of λ determined from Eq. (7).

EFFECT OF NONFIBROUS PARTICLES

In the RCF exposure experiments, the aerosol particles consisted of a significant fraction (56 to 72%) of nonfibrous particles which have an aspect ratio (length

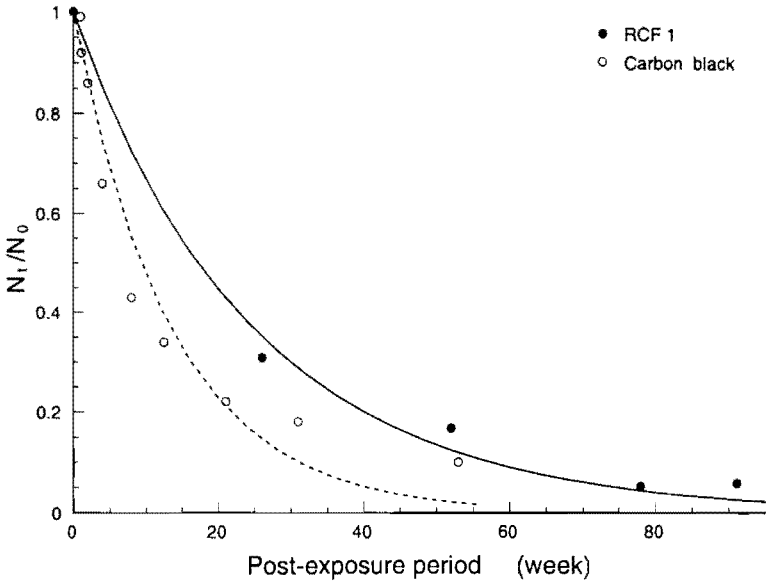

FIG. 4. Clearance curves of RCF 1 and carbon black from the rat lung at low lung burden. The full and open circles are data points from RCC and Strom *et al.* (1989), respectively. The predicted curves are shown by solid and dotted lines.

to diameter) of less than 3. Conceivably, these nonfibrous particles would affect the retention of RCF. The lung burden data of RCF shown in Fig. 3 do not include contributions from nonfibrous particles because this information is not available. To estimate the lung burden of nonfibrous particles during exposure and recovery postexposure, we used a deposition-clearance model for spherical particles with the clearance rate of nonfibrous particles given by Eq. (7). The equivalent geometrical mean diameter of the nonfibrous particles in the aerosol state was about 2 μm according to the size measurement.

In Fig. 5, we replot λ versus M for RCF, where M is the sum of the lung burdens of fibers and nonfibrous particles. The dependence of λ on M shown in this figure is qualitatively similar to the pattern shown in Fig. 3. The new correlation equation for RCF gives $a = 0.0055$, $b = 0.355$, and $c = 0.695$.

CALCULATION OF LUNG BUILDUP

During exposure, Eq. (1) can be integrated over fiber size to give

$$\frac{dN}{dt} = \int_0^\infty \int_0^\infty r(d_f, l_f, t) dd_f dl_f - \lambda(M)N. \tag{8}$$

The first term on the right-hand side of Eq. (8) is the total fiber uptake to the alveolar region of the lung which can be calculated from a deposition model (Yu *et al.*, 1994). To check the accuracy of λ for RCF shown in Fig. 4, we substitute the correlation equation for λ into Eq. (8) and calculate N as a function of time for different exposure conditions and compare the results with the experimental data determined in the RCC study. Because the experimental data were recorded as fiber number per milligram of dry tissue in the accessory lobe of the rat lung, Eq. (8) was calculated based upon a lobar lung model of Schum and Yeh (1980). The

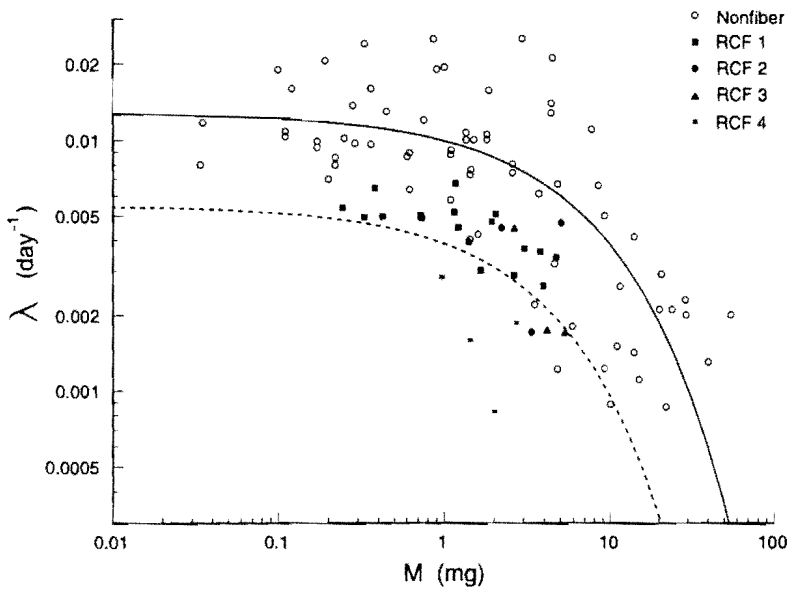

FIG. 5. Clearance rate λ versus M for RCF and nonfibrous insoluble particles. M of RCF includes both fiber and nonfibrous particle contributions.

dry/wet lung weight ratio was chosen to be 22% (Tillery and Lehnert, 1986). It was also assumed in the calculation that the lung volume (FRC) of a rat varied with the lung weight according to the following equation which we derived based upon the formula given by Stahl (1967):

$$\text{FRC} = 4.4\text{LW}. \qquad (\text{cm}^3) \tag{9}$$

In Eq. (9), LW is the lung weight of a rat in grams, given by Tillery and Lehnert (1986) to be

$$\text{LW} = 0.596 \ln W - 1.923, \qquad (\text{g}) \tag{10}$$

where W is the body weight in grams. The value of W was calculated from an empirical equation that we derived from the experimental body weight data of RCC, which gives

$$W = 206 + \frac{193t}{128 + t}, \qquad (\text{g}) \tag{11}$$

where t is the number of days of exposure. Equations (9) and (10) give a value of FRC close to that obtained from the formula of Stahl (1967) but the dependence of FRC on W is weaker. The minute ventilation used in the calculation was determined from the data of Mauderly (1986) and a constant breathing frequency of 98 breaths/min was assumed.

Figure 6 shows the results predicted by the model and actual experimental data of lung burden for RCF 1 in terms of fiber number per milligram of dry tissue at four different exposure concentrations of 3, 9, 16, and 30 mg/m^3. Comparisons between model prediction and experimental data show good agreement for all cases. To illustrate the effects of exposure concentration and altered clearance

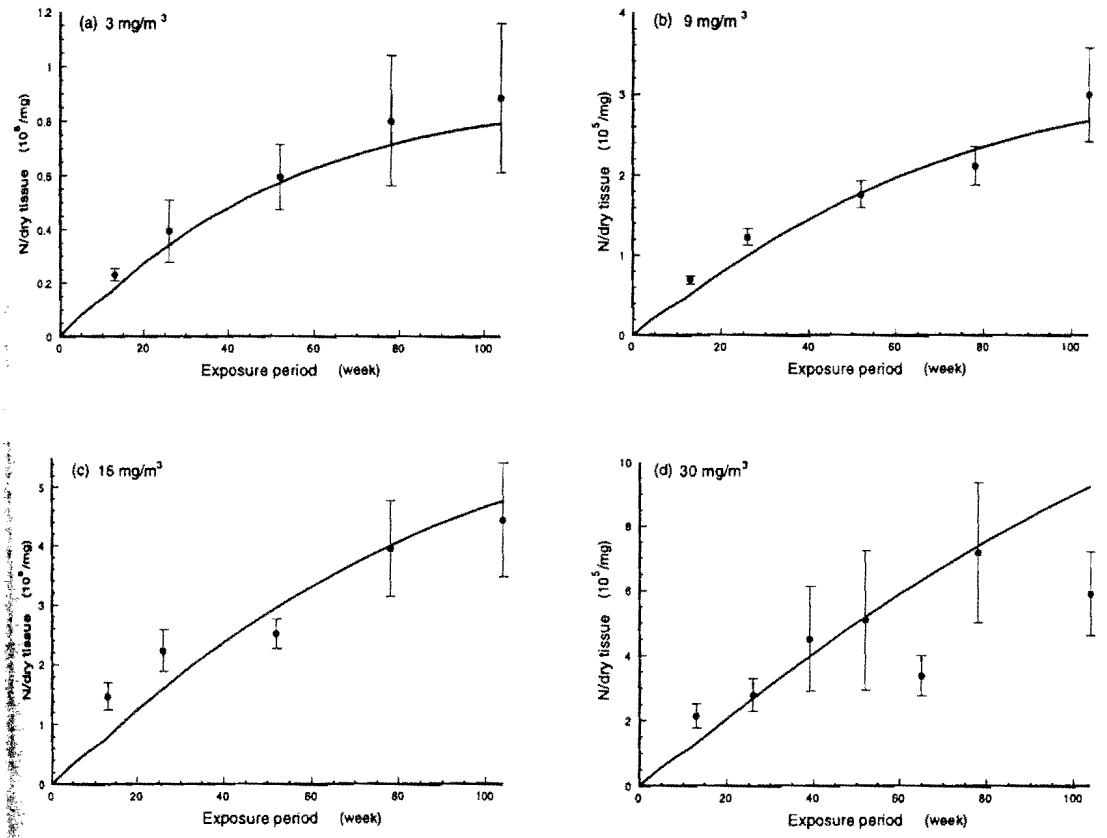

FIG. 6. Variations of number of fibers in milligrams of dry tissue with time for RCF 1 at four different exposure concentrations: (a) 3 mg/m^3, (b) 9 mg/m^3, (c) 16 mg/m^3, and (d) 30 mg/m^3. Solid lines are model predictions and full circles represent experimental data points.

rate λ (Fig. 3) on lung burden buildup, we normalized in Fig. 7 the lung burden results shown in Fig. 6 to the inhaled fiber number concentration. If there were no change in clearance rate with increased RCF burden, the normalized curves should all fall on the same line. However, as can be seen from Fig. 7, this is not the case. On a per unit concentration basis, the fiber number in the lung builds up faster at higher concentrations due to slower clearance rates.

CONCLUSIONS

Based upon available experimental data, we have developed a clearance model of refractory ceramic fibers from the rat lung. An expression for the clearance rate as a function of lung mass burden was derived. The following conclusions can be derived from our findings:

(1) compared to nonfibrous particles, lung fiber clearance rates are significantly greater for RCF (see Figs. 3 and 5);

(2) there does not appear to be a strong preference for clearance of a specific fiber size; and

(3) similar to nonfibrous particles, lung clearance of RCF decreases with increasing lung burden.

This last conclusion has implications for the appropriate choice of the dose–response model to be used for purposes of risk assessment. At elevated doses, this

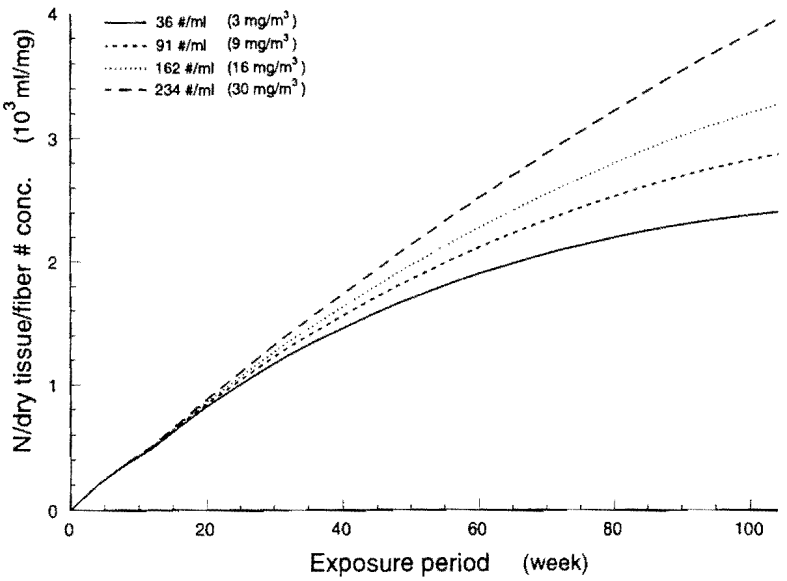

FIG. 7. Normalized fiber number buildup per milligram of dry tissue for RCF 1 at four different exposure concentrations.

analysis concludes that the rate of RCF clearance will be retarded and that the fibers will be retained in the lung for a longer time. If the probability of lung tumor induction is a function of fiber residence time as well as of dose—a reasonable supposition, it follows that the dose–response function in rats will be nonlinear and convex (i.e., bending upward). Indeed, only the highest exposure concentration of the RCC rat study showed an increased lung tumor incidence, i.e., the empirical relation between the observed incidence of cancer and fiber concentration was nonlinear.

In published guidelines for carcinogen risk assessment, the U.S. Environmental Protection Agency (EPA, 1986) notes that "no single mathematical procedure is recognized as the most appropriate for low-dose extrapolation in carcinogenesis," but argues that "in the absence of adequate information to the contrary, the linearized multistage procedure will be employed." The results of this paper suggest that a simple linear dose–response model for the rat may be inappropriate—a conclusion not based merely upon the (lack of) "goodness-of-fit" of the empirical data to a linear model, which EPA correctly notes is "not an effective means of discrimination among models," but rather on a careful analysis of the kinetics of clearance of RCF from the rat lung. To what degree this also applies to the human lung where retention half-times for spherical particles are longer by a factor of 10 needs to be evaluated further.

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