Research Report TM/01/04
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Data were available from two recent NIOSH studies of rats exposed by inhalation to respirable quartz. Data from the first study (including lavageable and non-lavageable lung burdens, lymph node burdens, and PMN and AM numbers) were used to estimate the model parameters not obtainable from the earlier studies, using a numerical method combining nonlinear least squares and integration of differential equations.

The second study was used for model validation by comparing the model predictions with the data. Once validated, the model was used to predict the outcome of a 2-year inhalation experiment with DQ-12 quartz and to derive a no observed adverse effect level for quartz.

Finally, the model was extrapolated to humans using appropriate scaling rules. The model was also extended to describe the dose-response relationships, including the oxidant dose, anti-oxidant reaction, transcription factor switching, cell damage, and fibrosis

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CONTENTS

SUMMARY

Objectives: (a) To construct a physiologically-based mathematical model to describe the retention and clearance of respirable quartz (MIN-U-SIL 5); the inflammatory reaction; and the development of fibrosis. (b) To calibrate/validate the model using animal data from inhalation experiments with MIN-U-SIL 5. (c) To use the model for extrapolation to lower concentrations and longer exposure durations.

Methods: A physiologically-based mathematical model was constructed with the following features: compartments to describe the retention and clearance of quartz; a threshold burden for initiation of inflammation; and a description of neutrophil (PMN) and alveolar macrophage (AM) recruitment. This is the first model to go beyond the earlier models (e.g. Tran *et al*, 2000) of retention and clearance of dusts to describe the exposure, dose, and response relationships. Data were available from two recent NIOSH studies of rats exposed by inhalation to respirable quartz (MIN-U-SIL 5, MMAD 1.6 μm) at 15 mg/m^3 for up to 24 weeks. Data from the first study (including lavageable and non-lavageable lung burdens, lymph node burdens, and PMN and AM numbers) were used to estimate the model parameters not obtainable from the earlier studies, using a numerical method combining nonlinear least squares and integration of differential equations. The second study was used for model validation by comparing the model predictions with the data. Once validated, the model was used to predict the outcome of a 2-year inhalation experiment with DQ-12 quartz (Muhle *et al*, 1991) and to derive a no observed adverse effect level for quartz. Finally, the model was extrapolated to humans using appropriate scaling rules and extended to describe the dose-response relationships, including the oxidant dose, anti-oxidant reaction, transcription factor switching, cell damage, and fibrosis.

Results: A good fit of the model to the data was obtained. Threshold burdens of MIN-U-SIL 5 were calculated for the initiation of both inflammation (0.20 mg $\pm$ 0.19) and fibrosis (1.96 mg $\pm$ 0.12). The model was able to simulate the long-term retention and clearance of DQ-12 quartz, suggesting similar biological mechanisms with both MIN-U-SIL 5 and DQ-12. The extended model describes the time course of NF-κB initiation, lipid peroxidation, and superoxide dismutase, although data are not yet available for validation of this portion of the model.

Conclusions: This physiologically-based kinetic lung model provides a means to relate the inhaled quartz dose to pulmonary inflammation and fibrosis. With appropriate interspecies scaling, this model potentially can be extrapolated to humans.

1. INTRODUCTION

The aims of the proposed collaborative study are:

(i) To develop a biologically-based mathematical model describing the retention and clearance of silica in the lungs and lung-associated lymph nodes of rats. Determine if there is a critical dose of silica in the lungs that influences the clearance rate.

(ii) To develop a biologically-based mathematical model describing the retention and clearance of silica in the lungs and lung-associated lymph nodes of humans.

(iii) To extend the model to describe the exposure-dose-response at the level of the oxidant dose, the anti-oxidant defence and cell damage.

1.1. RAT MODEL

1.1.1 'Low Toxicity Dust' model

The starting model for this project is the 3-compartment mathematical model derived for coalmine dusts. The inputs to the model are the factors that affect the deposited dose. The model predicts lung and lymph burdens dynamically for any given exposure scenario. The model can predict burden (lung, lymph nodes) as mass or surface area, given the specific surface area for the inhaled dust.

1.1.2 Rat Inhalation experiments

Data is available from two NIOSH experiments using MIN-U-SIL 5, one in 1997 the other, 1999. Lung (lavageable), interstitial (non-lavageable), lymph nodes and PMN were available for 1999, Lung and PMN for 1997. In the 1999 experiment, rats were exposed for 28, 54 and 84 days, at 15 $mg.m^{-3}$, each with a 0 and 36 day recovery group. In the 1997 experiment, rats were exposed at 7 times up to 161 days at 15 $mg.m^{-3}$. The experimental data , together with the exposure regimen, will be collated and summarised (means, SEs, group sizes) prior to modelling.

1.1.3 Calibration

Before extending the 'Low Toxicity Dust' model to quartz, it is necessary that the model be modified to the necessary number of compartments without loosing its physiological base. Furthermore, in extrapolating to humans, a model with less parameters will be more attractive as this will reduce the uncertainty in the modelling.

The factors affecting deposited dose will be summarised for each quartz experiment will be used to predict alveolar, interstitial and lymph burden and numbers of PMN for each of the experiments described in 1.1.2.

It is anticipated that this model will not fit the quartz data well, quartz being more highly toxic per unit surface area than coalmine dusts. Therefore, the parameter set will be calibrated using the observed data sets (the '99 experiment dataset) . Not all the model parameters will be included in this calibration.

A numerical routine (written in MATLAB) will be developed for parameter estimation. This method will combine non-linear least squares the Runge-Kutta algorithm for solving differential equations.

Data from the '97 dataset will be used subsequently to validate the model predictions. Because of the extra data available only in the '97 dataset (i.e. information on fibrosis, NF-κB, Lipid peroxidation etc...) , the model will be extended to describe the time course of these assays. However, this part of the model will need more data for validation.

1.2 SUMMARY STEPS FOR MODEL CALIBRATION/VALIDATION

1.2.1 Animal model

(i) 'Low Toxicity Dust' model will be reduced to minimal acceptable number of compartments without loosing physiological base.

(ii) Calibrate this model using NIOSH '99 data.

(iii) Validate this model using NIOSH'97 data.

(iv) Extend the model to describe the molecular exposure-dose-response relationship.

1.3 EXTRAPOLATION TO HUMANS

(v) Extrapolate the animal model to humans by:

- Changing the deposition parameters with the human equivalents;
- Replacing the (rat-based) parameters with the human (allometrically scaled) parameters.

2. AN EXPOSURE-RESPONSE MODEL FOR SILICA

2.1 THE EXPERIMENTAL DATA

The data were from an inhalation experiment with Crystalline Silica (Minu-Sil 5, MMAD=1.62 μm (gsd=0.12; high=1.86, low=1.47)). Male Fischer 344 rats were exposed at 15 mg.m^{-3} for up to 84 days. Groups of 8 rats were sacrificed at 28, 56 and 84 days during exposure. For each exposure group, a corresponding group of 8 rats were left to recover for 36 days before sacrifice. The following assays were measured at each of these time-points:

- Lavageable lung burden,
- Non-lavageable lung burden,
- Lymph node burden,
- Differential cell count from the Bronchio-Alveolar Lavaged (BAL) fluid:
- Number of Alveolar Macrophages(AM),
- Number of Neutrophils (PMN).

2.2 THE EXPOSURE-DOSE MODEL

An exposure-dose model was originally developed for coalmine dust (Kuempel *et al*, 2001a). This model was originally tested with human data (Tran and Buchanan, 2000). The model consisted of 3 compartments:

- X_1=The dust burden (mg) in the alveolar region,
- X_2=The dust burden (mg) in the interstitium,
- X_3=The dust burden (mg) in the lymph nodes.

The starting point for developing a mathematical model, describing the retention and clearance of silica in the rat lung, was to use this model (Figure 2.1). However, because silica was known to impair AM function, it was necessary to extend this model to include a compartment representing the silica burden in AMs. This model extension brought the current model closer to an earlier model developed for 'Low Toxicity' dusts (Tran *et al*, 1999; Tran *et al*, 2000).

Mathematically, the exposure-dose model is described by a set of differential equations:

$$\begin{aligned}
\frac{dX_1}{dt} &= Dose - r.X_1 - k_i.X_1 + k_d.X_2 \\
\frac{dX_2}{dt} &= r.X_1 - k_t.X_2 - k_d.X_2 \\
\frac{dX_3}{dt} &= k_i.X_1 - k_l.X_3 \\
\frac{dX_4}{dt} &= k_l.X_3
\end{aligned} \qquad (2.1)$$

where X_1 represents the silica burden in deposited in the alveolar region,

X_2 represents the silica burden inside AMs,

X_3 represents the silica burdens in the interstitium and

X_4 represents the silica burdens in the lymph nodes.

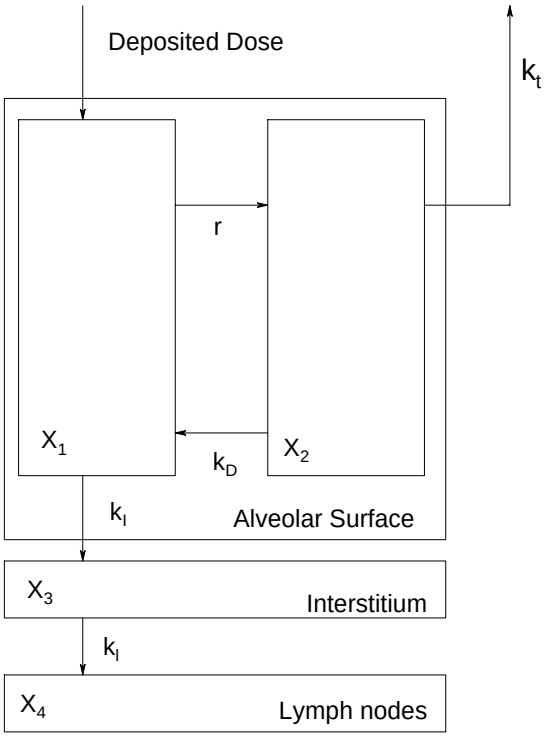

Figure 2.1 The schematic diagram of the exposure-dose model and the model parameters

Note that, with the onset of inflammation, neutrophils will also be present in the alveolar region. These cells were known to be phagocytosing particles (Donaldson *et al*, 1988). However, PMNs are very short-lived (life-span less than a day). Once dead, PMNs are quickly taken up by macrophages. Therefore, macrophages are the main cells for ingesting and removing silica particles (either as free particles or particles inside decaying PMNs). Thus, to keep the modelling simple, a compartment representing the silica burden inside PMNs was not included.

Dose is the deposited dose of silica and is calculated as:

$$Dose = \text{FD.conc.VI.DE.HE.CONV} \qquad (2.2)$$

where FD is the Deposited Fraction,

conc is the mass concentration ($mg.m^{-3}$),

VI is the volume of air inhaled (litre/min),

DE is the number of days per week exposed,

HE is the number of hours per day exposed,

CONV is the conversion into units of m^3 per day.

The other parameters are:

r the pagocytosis rate (day^{-1}),

k_i the interstitialisation rate (day^{-1}),

k_d the decay rate of AMs (day^{-1}),

k_t the AM-mediated clearance rate (day^{-1}),

k_l the translocation rate to the lymph nodes (day^{-1}).

The AM-mediated defence of the alveolar region is effective (i.e. phagocytosis and clearance of silica particles by AMs are unimpaired) as long as the silica burden inside AMs (X_2) remain below a critical burden level (m_{crit}). Once m_{crit} is reached clearance quickly becomes impaired and an inflammatory reaction is switched on. This impairment is modelled as:

$$F = 1 \qquad for\ X_2 \le m_{crit}$$
$$else \qquad (2.3)$$
$$F = e^{-B.\left(\frac{X_2 - m_{crit}}{m_{max} - m_{crit}}\right)^G}$$

and has been used to model the impairment of clearance in the earlier model (Kuempel *et al*, 2001a,b).
m_{max} is the maximum level of silica burden such that when $X_2 = m_{max}$, the impairment F will be e^{-B} (i.e. determined by B). B and G are parameters governing the speed of the impairment.

The impairment is assumed to affect the clearance of silica particles by AMs and the rate of release of silica from decaying AMs back to the alveolar surface (Tran *et al*, 1999).
Mathematically, the parameters k_t and k_d are made to be dependent on m_{crit} by writing k_t and k_d as $F.k_t$ and $F.k_d$.

2.3 THE DOSE-RESPONSE MODEL

A recruitment of PMN cells to the affected area of the alveolar surface occurs when free silica particles come into contact with alveolar epithelial cells and become interstitialised. This inflammatory reaction is in accordance with the results of 'low toxicity' dusts (Tran *et al*, 2000). However, a second source of PMN recruitment comes from activated AMs once m_{crit} is reached. This second inflammatory reaction is due to the effect of silica particles on AMs and has not been observed with 'low toxicity' dusts. The PMN recruitment process is modelled as:

$$\frac{dPMN}{dt} = k_1.k_i.X_1 + k_2.(1-F).X_2 - k_3.PMN \qquad (2.4)$$

The first term on the right hand side of eqn (2.4) represents the first source of PMN recruitment which is proportional to the interstitialisation rate (k_1 being the constant of proportionality). The second term is the second source of PMN recruitment. This is dependent on the burden of silica inside AMs and is switched on when m_{crit} is reached. The third term is the disappearance rate of PMNs (being taken up by AMs after undergoing apoptosis/necrosis).

k_1 constant of proportionality (in units of number of PMNs per mg of silica),

k_2 first order kinetics parameter (in units of number of PMNs per mg of silica per day),

k_3 disappearance rate of PMNs (in units of day^{-1}).

Interestingly, from the data the AM population did not appear to change during exposure and at post-exposure in comparison to the control AM population (ie.the AM population is in a steady state). Thus,

$$\frac{dAM}{dt} = 0 \tag{2.5}$$

2.4. METHOD FOR MODEL CALIBRATION

The model does appear to be over-parameterised at a first glance. However, the majority of the parameters have already been derived from earlier studies (Tran *et al*, 2000; Kuempel *et al*, 2001a,b). The strategy for model calibration is to estimate only the parameters which are suspected of being dependent on the nature of silica particles.

2.4.1 Fixed Parameters

The fixed model parameters and their values are given in Table 2.1.

Table 2.1 The fixed parameters of the exposure-response model

Parameters related to *Dose*		
Parameters	***Value***	***Units***
FD	0.06	Unitless
Conc	15	mg.m^{-3}
VI	0.18	litre/min
DE	0.714	days
HE	6	hrs/day
CONV	0.06	unitless
Parameters related to the exposure-dose model		
r	4.0	day^{-1}
k_d	0.033	day^{-1}
k_t	0.015	day^{-1}

Parameters related to the impairment function *F*		
B	6.9	unitless
G	0.7	unitless
Parameters related to the dose-response model		
k_3	0.01	day^{-1}

2.4.2 Parameters to be estimated

There are five parameters to be estimated. They are:

i)	k_I	the interstitialisation rate,
ii)	k_l	the translocation rate to the lymph nodes,
iii)	m_{crit}	the critical lung burden of silica.
iv)	k_1	the parameter relating to the first source of PMN recruitment,
v)	k_2	the first order kinetics related to the second source of PMN recruitment.

To estimate 1,2 and 3 we shall used the data from the lavageable lung burden, the non-lavageable lung burden and the lymph node burden. To estimate 4 and 5 we shall use the PMN data.

2.4.3 Method for parameter estimation

The model is written in the language of MATLAB. Subroutines from the MATLAB libraries (the toolboxes) were used to combine the numerical integration of the system of differential equations with non-linear least squares method for parameter estimation. Appropriate confidence intervals were also calculated.

The strategy is to estimate the parameters related to the exposure-dose model (1,2 and 3) first then estimate the parameters related to the dose-response model next (4 and 5). In all cases, the mean value of the data is used in the parameter estimation process.

The routines used were: *ode45* for solving the model numerically, *nonlinfit* for non-linear least squares and *nlpredci* plus *nlparci* for the parameter confidence interval estimations.

2.5 RESULTS

Table 2.2 shows the results of the parameter estimation process. Figure 2.2 shows the model predictions and the experimental data.

Table 2.2 Estimates for k_i, k_l , m_{crit}, k_1 and k_2 and their corresponding confidence intervals

	k_i	k_l	m_{crit}	k_1	k_2
Lower 95pc Estimate	0.0000	0.0000	0.0234	2.5780	0.2134
Estimate	2.1910	0.0042	0.2079	9.4072	0.3423
Upper 95 pc Estimate	4.7642	0.0196	0.3925	16.2365	0.4711

Final sum of squares for lavageable alveolar burden, non-lavageable alveolar burden and Lymph node burden are 0.0107, 0.0171 and 0.0031 respectively. The final sum of squares for PMN is 1.9391.

The model is now fully identified. In the next Chapter this model will be validated and extended with experimental data from a similar experiment.

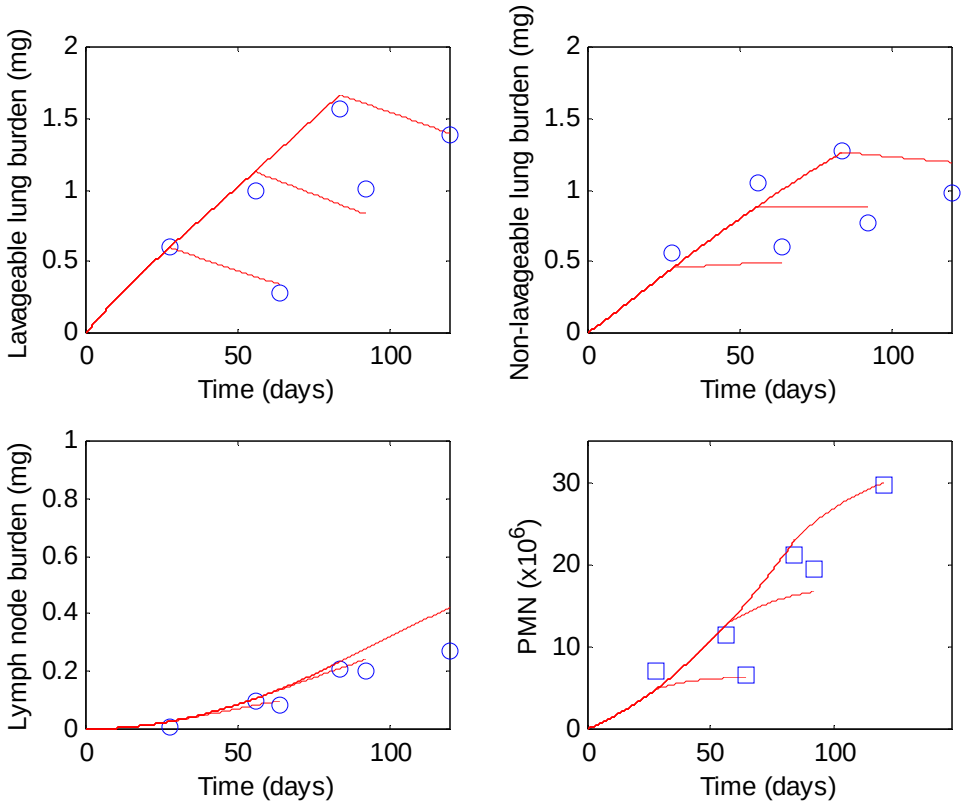

Figure 2.2 Model predictions and experimental data for (a) Lavageable lung burden; (b) Non-Lavageable lung burden; (c) Lymph node burden and (d) Number of Neutrophils in the BAL fluid

3. MODEL VALIDATION

3.1 EXPERIMENTAL DATA

The data used to test the model developed in Chapter 2 were from an inhalation experiment with the same silica type (Minu-Sil 5). Groups of 6 rats were exposed at 15 $mg.m^{-3}$ for up to 161 days. Sacrifice time points were at 1, 7,14, 28, 56, 112 and 161. The following assays were measured:

- Total burden (lung plus lymph node burden),
- Number of PMN cells,
- Number of AM cells,
- Hydroxyproline level (mg/lung) at 28, 56, 112 and 161 days respectively.

Note that the Hydroxyproline assay was not measured in the last experiment and therefore this assay wasn't part of the current model. However, the model was subsequently extended to include this assay.

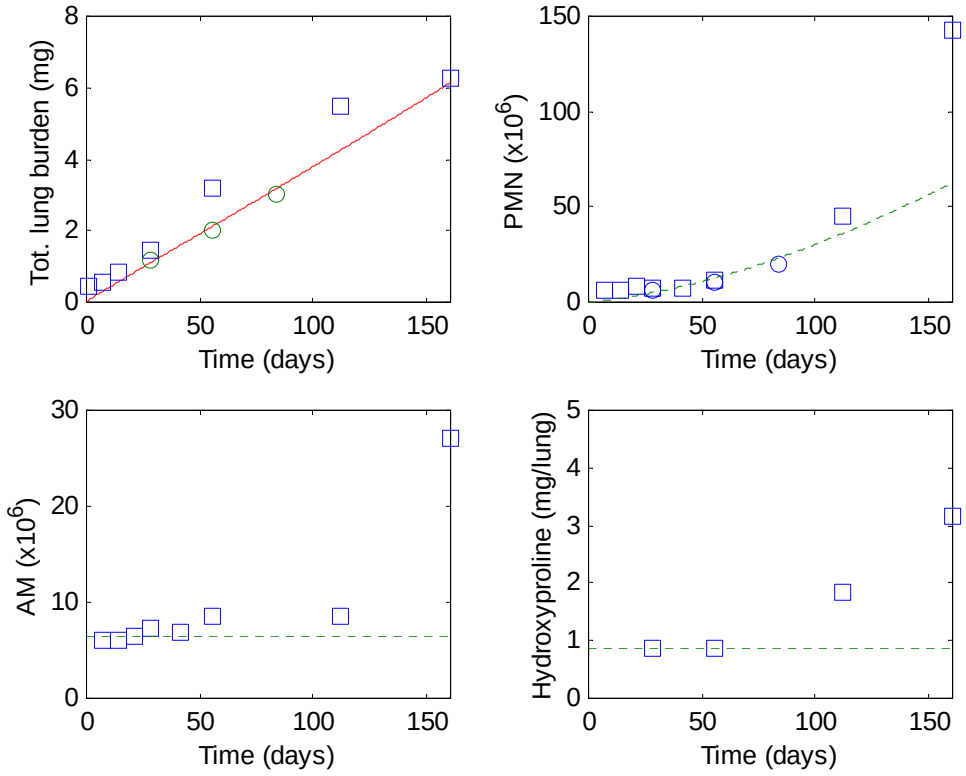

Figure 3.1 Model prediction and experimental data. The circles are data from the experiment in Chapter 2

Figure 3.1 shows the model predictions and the experimental data. Since the AM-mediated clearance became impaired at very low lung burden, the model predicted a linear build up of total lung burden. This appears to be confirmed by comparing the predicted total lung burden with the burden data of this experiment.

Interestingly, for the PMN, AM assays, the model under-predicted significantly the last time point (116 days). For Hydroxyproline, the level appeared to increase from 112 days. It is clear that

- The current model could not predict the formation of fibrosis and
- fibrosis worsened inflammation (i.e. increased PMN recruitment),
- fibrosis is associated with the increase in the AM population.

Finally, the formation of fibrosis appeared to occur once a threshold is reached.

3.3 MODELLING FOR FIBROSIS

A modified model to describe the occurrence of fibrosis and its effects was subsequently introduced. It was assumed that once the second threshold in X_2 (m_{crit_2}) was reached, AMs became seriously damaged such that extra recruitment of AMs was required and the phagocytosis function of AMs was also impaired leading to increased interstitialisation of free silica particles to cause fibrosis.

Mathematically, equations (2.4), (2.5) and (3.1) was extended with the use of a second threshold function F_2.

$$\begin{aligned} &F_2 = 1 \qquad\qquad for\ X_2 \le m_{crit_2} \\ &else \\ &F_2 = e^{-B.\left(\frac{X_2 - m_{crit_2}}{m_{\max} - m_{crit_2}}\right)^G} \end{aligned} \tag{3.1}$$

where B, G and m_{max} are given in Chapter 2 and $m_{crit_2} \ge m_{crit}$.

Equation (2.4) was extended as

$$\frac{dPMN}{dt} = k_1.k_i.X_1 + k_2.(1-F).X_2 + k_4.(1-F_2).X_2 - k_3.PMN \tag{3.2}$$

Similarly for equation (2.5),

$$\frac{dAM}{dt} = k_d.AM_0 + k_6.(1-F_2).X_2 - k_d.AM \tag{3.3}$$

and equation (3.1)

$$\frac{dH}{dt} = k_5.(1-F_2).X_2 \tag{3.4}$$

Finally, the phagocytosis rate, r, was made to be dependent on F_2. i.e.

$$r = r_0.F_2 \tag{3.5}$$

where r_0 is the value of r used in the original model (see Chapter 2).

3.4 RESULTS

The re-modelling process introduced four extra parameters, namely, m_{crit_2}, k_4, k_6 and k_5. Using the method described in 2.4.3., the estimates for the parameters were obtained.

Table 3.1 The parameter estimates and their upper and lower 95 pc confidence values

	m_{crit_2} (mg)	k_4 (day^{-1})	k_5 (day^{-1})	k_6 (day^{-1})
Lower 95pc Estimate	1.848	1.158	0.012	0.373
Estimate	1.964	1.267	0.029	0.505
Upper 95pc Estimate	2.080	1.376	0.047	0.640

The extended-model predictions and the experimental data are shown in Figure 3.1.

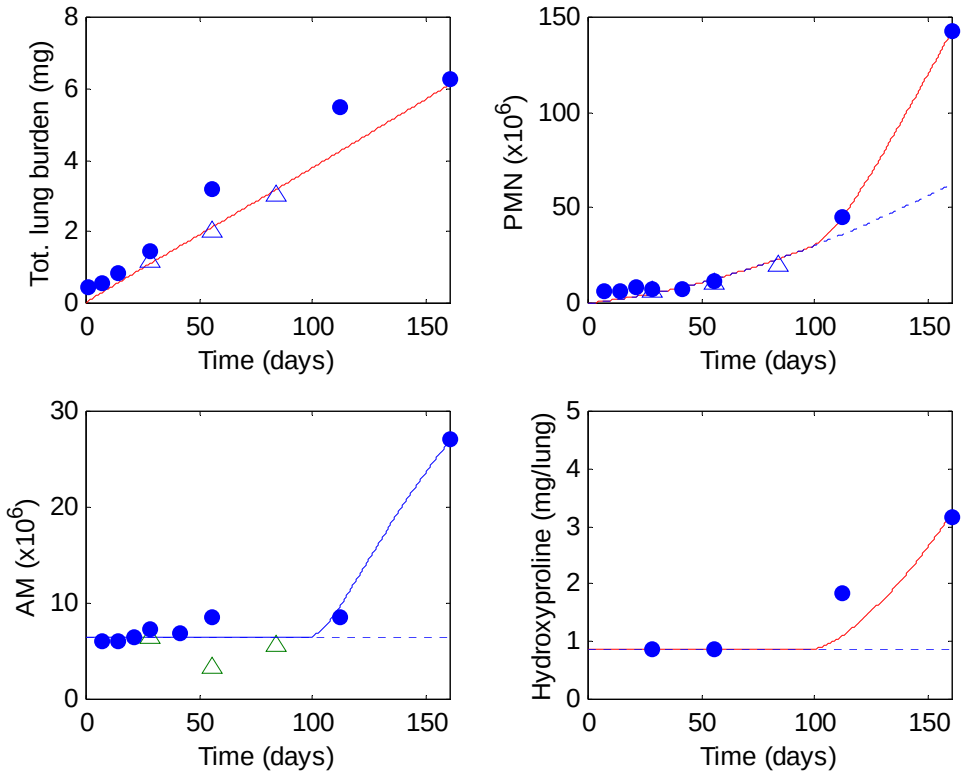

Figure 3.2 The extended-model predictions and the experimental data from the second inhalation experiments with Minu-Sil 5. The triangles are data from the first experiment described in Chapter 2; the dotted lines are the original model predictions

4. MODEL EXTRAPOLATION

4.1. METHOD

Using the parameters estimated using the '99 experiment data, the model was used to extrapolated to a low concentration situation. Data were available from a chronic 2-yr study with SiO_2 (MMAD=1.4 μm, gsd=1.8, DQ-12 quartz; Muhle *et al*, 1991) at 0.75 mg.m^{-3}. The data consist of lung and lymph node burden for up to 2 years and one time point for 1.5 month after 2 years exposure.

i. Use the model to predict the Muhle experiment;
ii. Change some parameters to make model to fit data if necessary.

Because of different particle size distribution, different silica type and different experimental conditions, it is expected that the deposition fraction and the translocation rate to the lymph nodes to be different.

The deposition fraction for the Minu-Sil 5 is 0.06 (see Chapter 2). Using the data from Raabe *et al* (1977, 1988), the deposition fraction for the DQ-12 quartz was estimated to be 0.08 (see Figure 4.1).

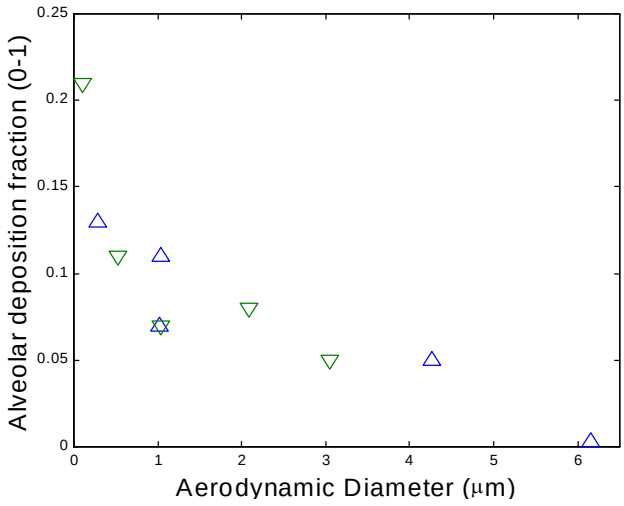

Figure 4.1 Alveolar deposition fraction for particles inhaled by rats. Data from Raabe *et al*., 1977 (Δ) and 1988 (∇)

4.2. RESULTS

Figure 4.2 show the model simulations. The dotted lines represent the model simulations using the original parameters estimated in Chapter 2.

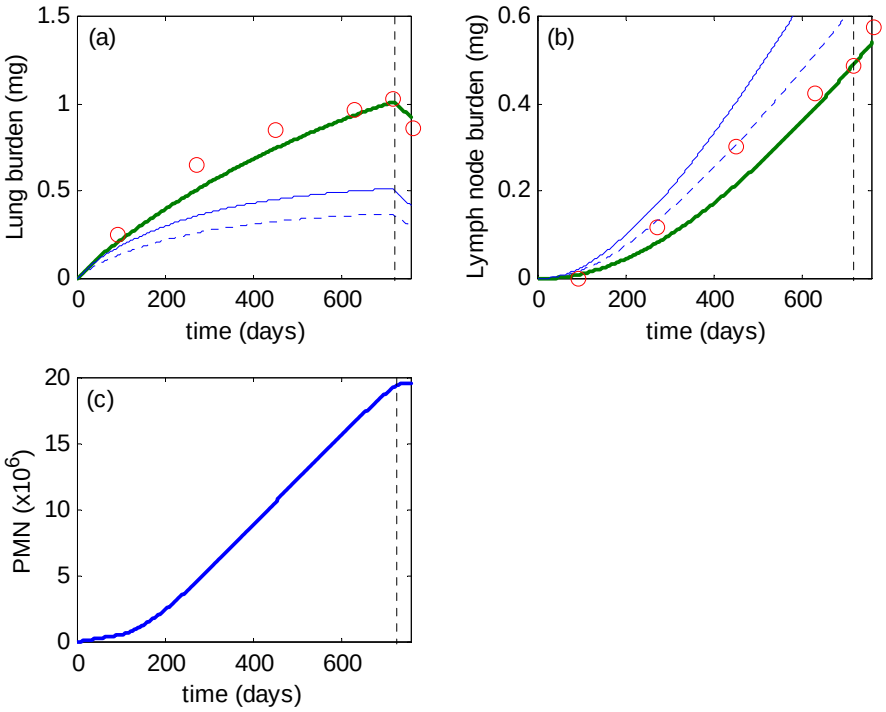

Figure 4.2 Model predictions for a 2-yr chronic inhalation study with DQ-12 quartz at 0.75 mg.m^{-3}. (a) Lung burden; (b) Lymph node burden; (c) PMN level

The solid lines are obtained using a value of deposition fraction *DF* of 0.087 and a translocation rate k_l of 0.0015 (day^{-1}). These values were obtained 'by eyes', since the objective is to demonstrate the consistency in model predictions. The vertical line represents the end of the exposure period. The light solid lines are the simulations with *DF* = 0.08 and k_l = 0.0042 (the original value from Chapter 2).

Note that the estimated value of k_l is still within the 95 percent confidence interval of the estimated k_l using the '99 experimental data (see Chapter 2).

4.3. NO ADVERSE EFFECT LEVEL (NAEL)

The adverse effect considered in this Chapter is inflammation as represented by the number of PMN in the BAL fluid. Specifically, the criterion for a 'no adverse effect level' used in this exercise is the concentration level such that 'the PMN response is lower than the average of control level minus twice the standard error as found in the '97 experiment' (see Chapter 3). From this data, the average PMN level in control groups was calculated as 1.28x10^6 (2 x ste 1.8 x 10^6).

In this section, the extrapolation exercise is taken one step further. Using the DQ-12 results as starting point, the model is used to simulate the exposure-dose-response relationship at concentration levels lower than 0.75 mg m^{-3}. Starting at a concentration level of 0.5 mg.m^{-3} and reducing the concentration at a step of 0.1 a time until 0.1 mg.m^{-3}. The concentration level that satisfies the definition of no adverse effect, described above, is 0.1 mg.m^{-3}. This level is incidentally the permissible level for quartz in the US. The corresponding simulations of the exposure-dose-response are shown in Figure 4.3.

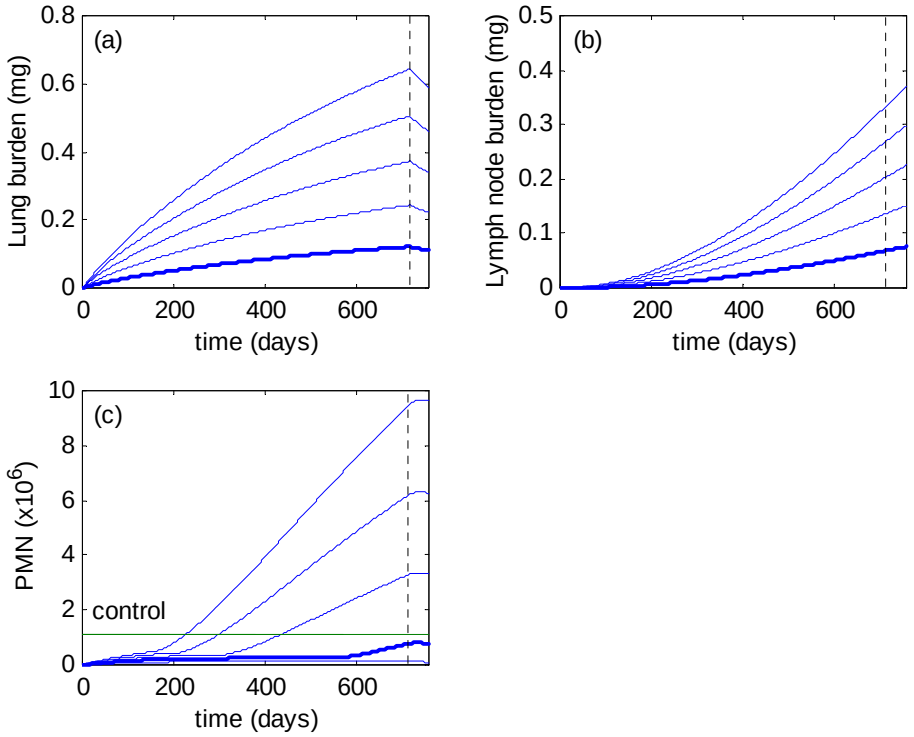

Figure 4.3 Extrapolations to lower concentration levels to derive a safe level for the average animal. The bold curves correspond to 0.1 mg.m^{-3}. (a) Lung burden; (b) Lymph node burden; (c) PMN level

4.4 EXTRAPOLATION TO HUMANS

The extrapolation exercise above illustrates a method for calculating a safe limit for humans using animal data. However, the model can also be used to extrapolate the results to human situations using (a) information available for humans whenever possible or (b) extrapolated values from animal data. In this section, a method for model extrapolation will be presented. The resulting human equivalent model will be used to simulate the exposure-dose-response relationship in humans following a working life-time exposure to silica at various occupational relevant airborne concentration levels.

Table 4.1 summarises the model parameters, currently identified using animal data, which will be extrapolated to humans.

Table 4.1 Model parameters to be extrapolated to human equivalents

Exposure	Deposited Dose	Retention and Clearance		Cell Recruitment	Fibrosis
Conc	*FD*	r		k_d	k_5
t_{start}	*VI*	k_t		k_1	m_{crit2}
t_{final}	*HE*	k_i		k_2	
	DE	k_l		k_3	
	CONV	m_{crit}		k_4	
		m_{max}		k_6	
		B	Eqn (2.3)	AM_0	
		G			

4.5 METHOD FOR EXTRAPOLATION

The following method was used in extrapolating (animal based) model parameters to their human equivalents.

(i) *for Exposure*

Replace the parameters with the relevant human occupational equivalents (i.e. 0.1 $mg.m^{-3}$).

(ii) *for Deposited Dose*

Use the formula in the human model (Kuempel *et al*, 2001 a,b).

(iii) for Retention and Clearance

Scale all rate parameters with respect to surface area (Ings, 1990):

e.g. $r_{human} = r_{rat}$. (lung surface area rat/lung surface area human)$^{0.25}$.

Scale m_{crit} and m_{max} as mg/g lung of rat then multiply by human lung weight to get absolute values for these parameters for humans.

vi) *for Cell Recruitment*

For k_d, assume life-cycle of rat AMs and PMNs are the same as humans.

AM_0=steady state of AM population. This value exists in literature for humans.

In the absence of all information all the recruitment rates are assumed to be the same as rats as a first guess. However, it could be argued that the recruitment of phagocytes and their removal are events which take place on the alveolar surface and therefore the extent of such events is very much dependent on the size of the surface area of the animal specie. In this exercise the recruitment rates were left unchanged.

vii) *for Fibrosis*

m_{crit2} is extrapolated in the same way as m_{crit}.

k_5 is assumed to be the same as the k_5 for rats.

4.6 RESULTS FROM EXTRAPOLATION

The results from the extrapolation exercise are shown in Figure 4.4. The animal based model parameters and their human equivalents are shown in Table.4.2.

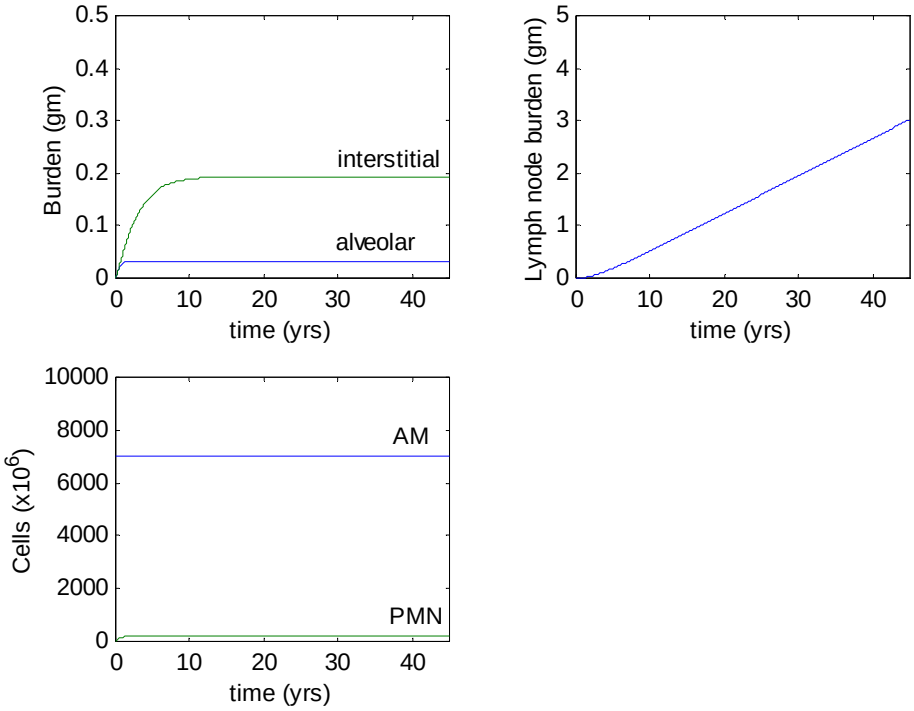

Figure 4.4 The exposure-dose-response relationship expected in humans exposed to silica at 0.1 mg.m^{-3} for 20 years. (a) Simulated burden in different compartments of the human deep lung; (b) the AM and PMN response

4.7 DISCUSSION

The deposition fraction used in this exercise came from literature (Raabe *et al*, 1977; 1988). A slight change to this parameter was made to bring the model in line with the data. The final estimate for this parameter is still in the range of the plausible values given the scattering expected from this dataset. The reduction in the parameter k_l, however, may be due to the experimental condition, method for measuring the burden or silica type. From Figure 4.1, it is clear that, if k_l is kept constant then to fit the (under-predicted) lung burden *FD* would have to be increased. This move will further increase the lymph node burden.

To conclude, the model is generally consistent across the range of silica data available. Importantly, the deposition of efficiency of silica particles can be derived from data on deposition. The translocation rate may be different for a number of reasons, however, the final corrected rate is arguably not too different from the original value derived from the Minu-Sil data. Most importantly, the rest of the parameters were left constant, especially, m_{crit}, the model have shown that this threshold was reached within the 2-yr inhalation period so inflammation is to be expected (Figure 4.1). It is interesting to note that Muhle *et al* (1991) also reported of incidences of cancer in some rats following exposure. The current model predicts inflammation (as PMN recruitment) following DQ-12 inhalation. The inflammatory response worsens after 200 days and is persistent throughout exposure. It may well be that this persistent level of inflammation in some rats had lead to cancer.

The model is subsequently used to extrapolate the exposure-dose-response relationship to humans. Applying the rule for scaling with respect to surface area for the parameter relating to the kinetics of retention and clearance while assuming that the cell kinetics are the same,

the model predicted a steady state in the build up of silica lung burden following a life-time exposure (45 years) to silica at 0.1 mg.m^{-3}. However, it is likely that the model, in its current set up, may under predict lung burden and over predict lymph node burden.

Table 4.2 The rat based model parameters and their human counterparts

Parameter	Rat	Human
Exposure		
Concentration (mg.m^{-3})	0.10	0.10
t_{start} (yr)	0.00	0.00
t_{end} (yr)	2.00	45.0
Deposited Dose		
FD	0.06	0.32
VI	0.18	13.5
HE	6.00	8.0
DE	0.714	0.714
CONV	0.06	1
Retention and Clearance		
r	4.00	0.9660
k_t	0.015	0.0036
k_i	2.19	0.5289
k_l	0.0042	0.0010
m_{crit} *(mg)*	0.21	1.468 x 10^2
m_{max} *(mg)*	10.0	10.0 x 10^2
B	6.9	same as in rats
G	0.7	
Cell Recruitment		
k_d	0.03	
k_1	9.40	
k_2	0.34	same as in rats
k_3	0.01	
k_4	1.267	
k_6	0.505	
AM_0	6.5 x 10^6	7 x 10^9
Fibrosis		
k_5	0.029	same as in rats
m_{crit2} *(mg)*	1.964	6.99 x 10^2
Lung parameters		
lung weight (gm)	1.43	1000
lung surface area (cm^2)	4865	143000

Tran and Buchanan (2000), working on data available from UK coalminers, estimated an average k_l (arithmetic mean) of approximately 1x10^{-4}, which is 10 times smaller than the current exptrapolated value of k_l. The human value for k_t is nearer to the often quoted value of 0.0015 (Kuempel *et al*, 2001 a.b). The model has predicted a high level of interstitial burden as compared to alveolar burden. This has been observed by (Kuempel *et al*, 2001 a,b) and may be characteristics of the human processes of retention and clearance.

The human PMN response predicted by the model is negligible comparing to the expected AM level. Currently, there is no equivalent human data to verify and validate this prediction.

5. MODELLING THE MOLECULAR DOSE-RESPONSE RELATIONSHIP

In this Chapter the exposure-dose-response relationship for silica will be explored further. It is commonly agreed that particles cause oxidative stress and that this is an important mechanism for the initiation of inflammation. In addition the inflammatory cells that migrate to the lung eventually contribute to the oxidative stress. Oxidative stress arises from lipid peroxidation products such as 4 hydroxynonenal (4HNE) causing depletion of glutathione (GSH), the major small molecular weight anti-oxidant of the epithelial surface. When this occurs there is 'sensing' of the oxidative stress with activation of redox sensitive transcription factors such as NF-κB and AP-1 that control the transcription of pro-inflammatory and proliferative genes (Rahman and MacNee, 1998).

There is little work on the molecular signalling events following silica exposure that lead to inflammation but there is clear evidence of oxidative stress at the silica surface (Donaldson and Borm, 1998) and activation of NF-κB (Sacks *et al*, 1998;). The steady state depletion of I-κB in silica-exposed cells very likely underlies the chronic switching on of IL-8. It is likely that oxidative interactions with the cells cause signalling that leads to inflammation that contributes to fibrosis and cancer.

A growing amount of evidence indicates that cytokines locally produced by inflammatory cells in the lung play a pivotal role in the control of a toxic response. In particular, the list of cytokines involved in the pulmonary response to inhaled particles is rapidly increasing and includes TNF-α, IL-1, IL-6, chemokines, IL-10, TGF-ß,…. In addition, recent studies show that cytokines affect fibroblast activation and proliferation, as well as collagen deposition during evolution of chronic fibrotic lung disease. In particular, γ-interferon suppresses fibroblast activities such as proliferation and collagen production, while interleukin-4 augments fibroblast growth and collagen production (Sempowski *et al*, 1996). These two mediators are the prototypic cytokines which functionally define either a Th1 or a Th2 response (Mossman *et al,* 1986). Clinical studies have also revealed a predominance of the Th2 cytokine pattern of inflammatory response in the pulmonary interstitium in fibrotic diseases (Wallace *et al,* 1995).

Recent findings indicate that anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGF-β) which accompany and tend to activate the resolution of the inflammatory response induced in the lung by inhaled particles, all belong to the TH2 cytokine group. This suggests that the sustained attempt by the lung to resolve the inflammatory reaction induced by silica particles could contribute to the pathogenesis of the fibrotic reaction through the protracted secretion of pro-fibrotic cytokines (Huaux *et al,* 1999).

The exposure-dose-response relationship for inhaled silica is summarised in Figure 5.1.

5.1 MATHEMATICAL MODELLING

In this Chapter, we shall attempt at modelling the exposure-dose-response relationship at the level of the oxidant dose, the anti-oxidant response, the activation of the transcription factor NF-κB and the oxidant-induced cell damage. This part of the modelling exercise is not as rigorous as the work in the preceding chapters because there is a lack of data for model calibration/validation. However, it is anticipated that the work presented in this chapter will lay the ground for future molecular dosimetric studies. Figure 5.2 gives a schematic diagram of the oxidant/anti-oxidant interaction.

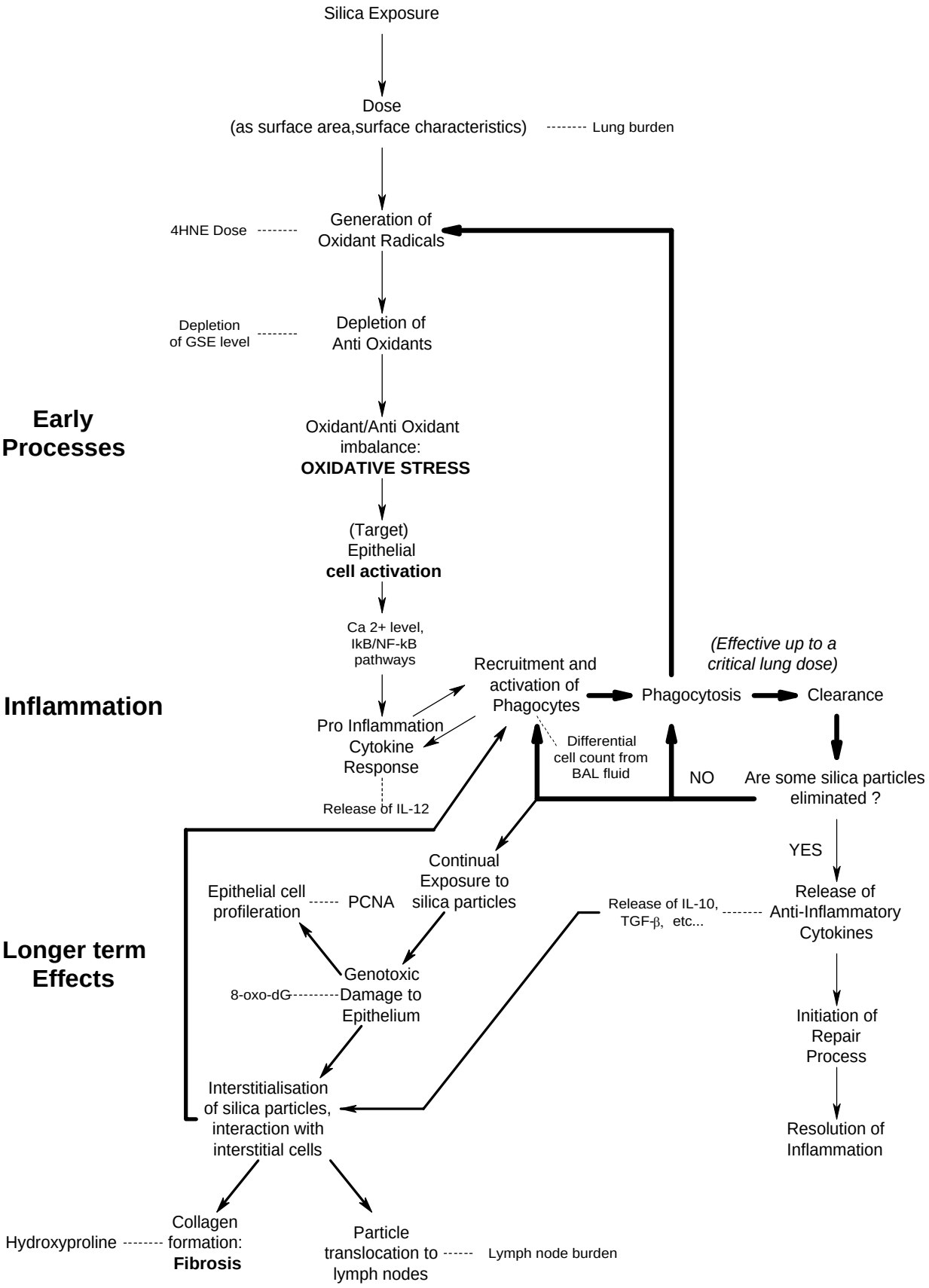

Figure 5.1 The exposure-dose-response to inhaled silica. The early processes leading to Inflammation and the longer term effects

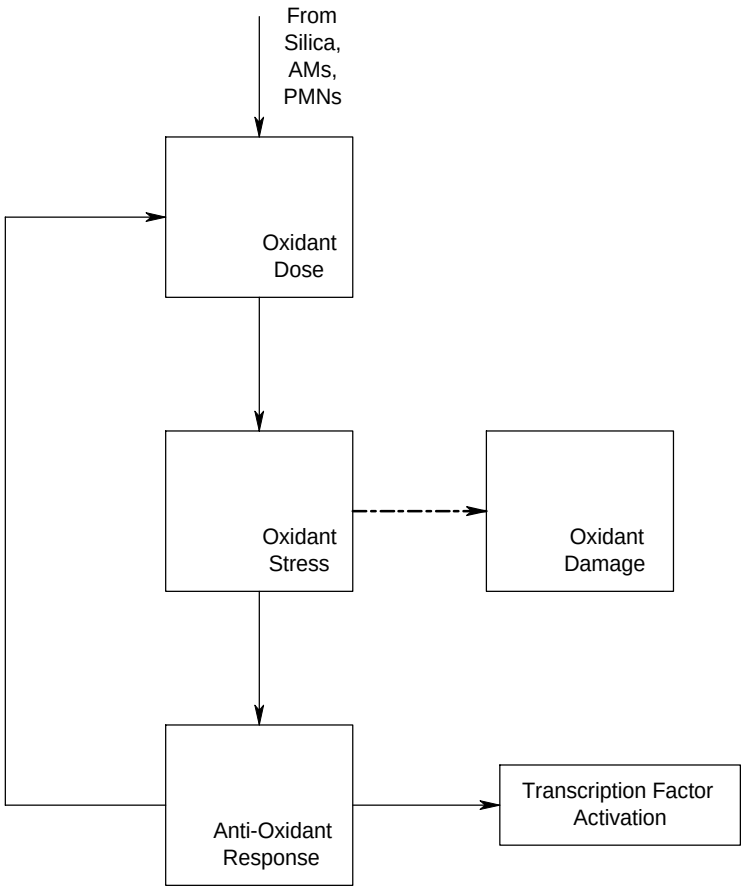

Figure 5.2 Schematic representation of Oxidant/Anti-Oxidant interaction

5.2 OXIDANT PRODUCTION

As a first step at modelling this interaction, let *O* be the anti-oxidant and *A* be its anti-oxidant counterpart. In the silica experiment, *O* is produced from *three* sources, namely, the silica deposited dose in the alveolar region, the macrophages and neutrophils.

We can describe the production of *O* as:

$$\frac{dO}{dt} = \lambda_1 D + \lambda_2 X_0\, AM + \lambda_3 X_0\, PMN - k_0\,(A - A_0) \qquad (5.1)$$

where

D is the deposited dose of silica in the alveolar region, as calculated in the model (mg/d).

λ_1 is the amount of *O* produced per unit of silica (nmol/unit mass).

λ_2 is the amount of *O* produced per *AM* (nmol/cell/unit mass)..

λ_3 is the amount of *O* produced per *PMN* (nmol/cell/unit mass)..

AM is the macrophage population at time *t* (x 10^6).

PMN is the neutrophil population at time *t* (x 10^6).

X_0 is the amount of free silica on the alveolar surface (mg).

The last term has to do with the amount of *O* eliminated by *A*.

Note that in a control situation (with silica exposure), Eqn (5.1) is reduced to

$$\frac{dO}{dt} = -k_0 (A - A_0) \tag{5.2}$$

where AM_0 is the residential macrophage population.

In the control situation $A = A_0$ so $\frac{dO}{dt} = 0$ and $O = O_0$.

5.3 ANTI-OXIDANT PRODUCTION

A is produced by the lung to neutralise *O*. However, the process of *O* neutralisation may be limited because of inefficiencies in:

- the ability of the lung to produce *A* and
- the amount of *A* required in neutralising an unit of *O*.

Without exposure to silica, the *A* level is in a steady state.

$$\frac{dA}{dt} = -k_0 (A - A_0) \tag{5.3}$$

where A_0 is the baseline level of *A*,

k_0 is the rate of *A* production (day^{-1}),

At the steady state level $A_0 = A$. d*A*/d*t* is zero. Therefore,

$A = A_0$ is a solution.

Now, when the lung is exposed to silica, an extra term is added to Eqn (5.3). This term represents the rate of extra *A* produced because of oxidative stress.

$$\frac{V_{max}}{1 + [k]/(OS - OS_0)} \tag{5.4}$$

This is the usual Michaelis-Menten expression. We use it because we assume that the production of *A* is rate limited.

where V_{max} is the maximum rate of *A* production.

[k] is the affine constant.

O_0 is the baseline oxidant level.

In this case, Eqn (5.3) becomes

$$\frac{dA}{dt} = \frac{V_{\max}}{1+[k]/(OS-OS_0)} - k_0\,(A-A_0) \tag{5.5}$$

Note that when OS is near to OS_0, Eqn (5.5) is reduced to Eqn (5.3).

In summary, Eqns (5.2) and (5.3) represent the steady state control situation. The ratio $\frac{O_0}{A_0}$ is the oxidant to anti oxidant balance in a control situation.

One implication of this model is that for large O, Eqn (5.5) tends to

$$\frac{dA}{dt} = V_{\max} - k_1\,(A-A_0) \tag{5.7}$$

which can be solved to give a steady state level of Anti-Oxidant A_{ss}

$$A_{ss} = \frac{V_{\max} + k_1 A_0}{k_1} \tag{5.8}$$

5.4 OXIDANT DAMAGE

The rate of Oxidative Stress (OS) is assumed to be proportional to the rate of oxidant production.

$$\frac{dOS}{dt} = \gamma_1 \frac{dO}{dt} \tag{5.9}$$

where γ_1 the constant of proportionality.

5.5 ACTIVATION OF TRANSCRIPTION FACTOR NF-κB

The transcription factor *NF-κB* is activated as the results of oxidative stress induced by the deposited silica particles on epithelial cells. Thus, we expect *NF-κB* production to be proportional to the anti-oxidant production:

$$\frac{dNF-\kappa B}{dt} = \gamma_2 \frac{dA}{dt} \tag{5.10}$$

where γ_2 the constant of proportionality.

5.6 MODEL SIMULATION RESULTS

From experimental data, for Anti-Oxidant, we measured Superoxide Dismutase (SOD). For Oxidative Stress we measured Lipid Peroxidation (LP). There is no data available for Oxidant but some estimates of the oxidant produced by alveolar macrophages can be obtained from *in vitro* data. The production of the transcription factor *NF-κB* was also measured.

The parameters for this part of the model are derived, either from *in vitro* experiments or derived by comparing 'by eyes' the model outputs with data. Table 5.1 gives a summary of the model parameters, their values.

Table 5.1 Model parameters used to obtain the model predictions in Figure 5.3

Parameters	Value	Source
	Steady States (Control)	
O_0	0.0	
A_0	120.0	*from data in control experiment*
OS_0	100.0	
$NF\text{-}\kappa B_0$	1.0	
	Exposure	
λ_1	0.0	*Assumed that silica is not freshly fractured,*
λ_2	2.8	*from in vitro data*
λ_3	3.2	
k_1	0.001	
k_2	0.005	*Estimated from inhalation experiment data*
V_{max}	4.0	
$[k]$	2.0	
γ_1	0.5	
γ_2	0.004	

5.7 DISCUSSION

Given the parameters in Table 5.1. The model predictions gave a reasonable description of the data (Figure 5.3). Currently we model the various threshold value for initiation of inflammation, fibrosis with respect to the silica mass inside macrophages. If the modelling of Oxidant and Anti-Oxidant is achieved (including parameter estimation, model validation) then the threshold in the current model should be changed to that of oxidant dose. The pulmonary responses can be described by (i) the activation of NF-κB, (ii) anti-oxidant regulation, (iii) cytokine released (data not available) and (iv) oxidant-induced cell damages. The current model is admittedly an over-simplified description of the relationships between these molecular events. However, this is the first step at linking various datasets, each representing events at different levels (e.g. cellular, molecular etc...). This modelling exercise has helped in arranging the description of various pathological pathways leading to disease (i.e. fibrosis) in a way which yields a clear and coherent view of the complex events, from the deposition of silica particles to the development of fibrosis.

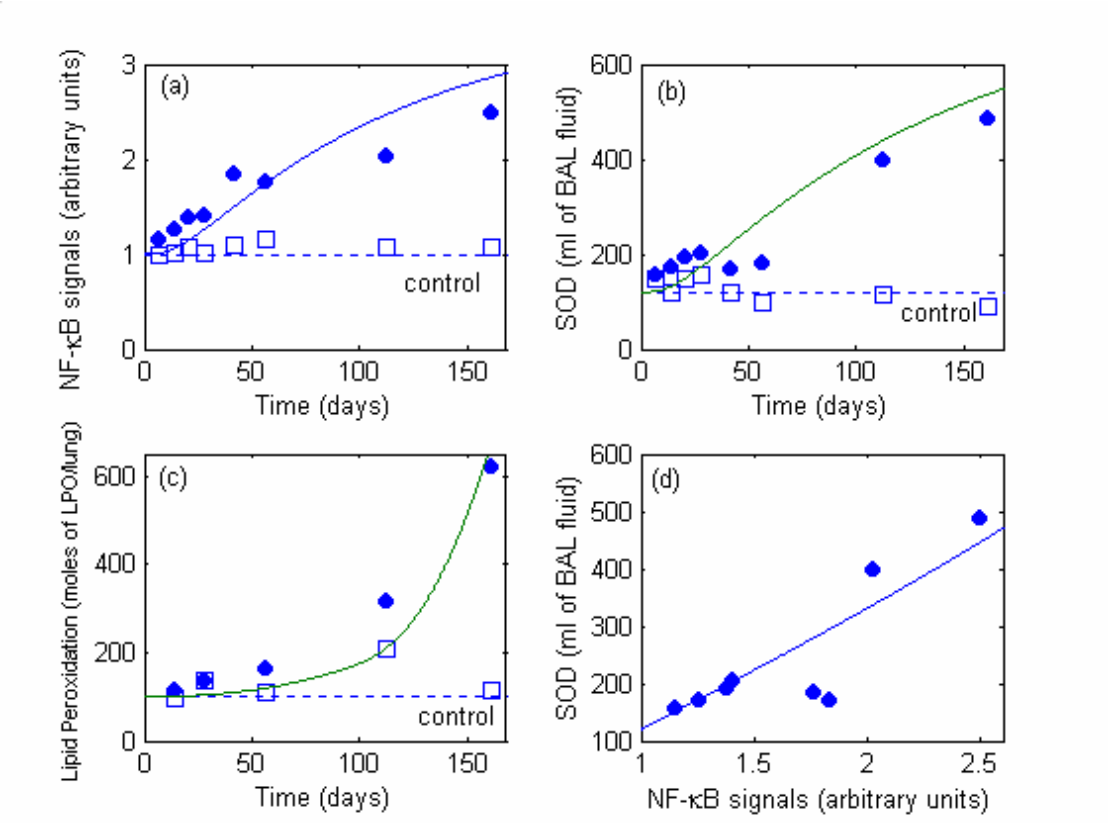

Figure 5.3 Time course of (a) NF-κB; (b) SOD; (c) LP; (d) the relationship between Inflammation (NF-κB) and Anti Oxidant response (SOD). (□ are data from control experiment)

6. DISCUSSION

Most mathematical models developed in the field of inhalation toxicology of particles and fibres described the retention and clearance of the deposited particulates in the pulmonary region of the lung. Stöber *et al* (1994) first considered the kinetics of alveolar macrophages (AM) and constructed a mathematical model based on the particle loading of macrophages. The underlying hypothesis of this model was the one put forward by Morrow (1994) on the volumetric overloading of AMs leading to the impairment of AM-mediated clearance. Tran *et al* (1994) constructed a similar model for the retention and clearance of quartz. This model used a similar mathematical description of the AM kinetics to that of Stöber but also included a description of the kinetics of neutrophil (PMN) recruitment and disappearance. These two models were the first to expand beyond a quantitative description of the particulate dose to describe the cellular responses. However, it was recognised that both models were over-parameterised and there was not enough relevant data available to validate the models fully. To reduce the number of model parameters, a smaller model of retention and clearance of inhaled respirable particles was developed (Tran *et al*, 1999a,b). A similar, but much reduced model was also used to describe the retention and clearance of coal mine dust in humans (Kuempel *et al*, 2001a,b). These two exposure-dose models formed the basis for the current modelling exercise.

In the current study, we have extended the exposure-dose model of Kuempel *et al* to include a macrophage burden compartment. This crucial compartment gave the basis for the threshold doses (m_{crit} and m_{crit2}) of crystalline silica activating inflammation then causing fibrosis. The sub-models of cellular responses (AM and PMN) were also added. Finally, the model was extended to cover tissue damage (Hydroxyproline burden), oxidative stress (Lipid Peroxidation), anti oxidant reaction and transcription factor activation (NF-κB). Although not all sub-models are currently validated, the model has offered a unique, quantitative and coherent view of the events leading to the development of fibrosis and their inter-dependence.

The model was successfully tested on an independent dataset (Muhle *et al*, 1991) and used to predict a No-Observed-Adverse-Effect-Level (NOAEL) for quartz. Using appropriate scaling rules, it was possible to extrapolate this model to a human based model. Due to lack of human data, it was not possible to verify the model predictions directly. However, the results from similar modelling studies (Kuempel *et al*, 2001a,b) suggest that the scaling rules are likely to over-estimate the kinetics of quartz translocation to the lymph nodes.

The current study has elucidated many aspects of the relationship between inhaled silica and the occurrence of fibrosis. Threshold doses of quartz were estimated and NOAEL calculated. These practical results will help inform and improve the risk assessment of exposure to crystalline silica.

7. ACKNOWLEDGEMENTS

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