Risk assessment of inhaled particles using a physiologically based mechanistic model

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There is a need in biological research to minimise the use of animal experimentation, but procedures to set exposure limits via the concept of the No Observed Adverse Effect Level (NOAEL) have traditionally needed large numbers of animals to investigate levels corresponding to low risks. Dynamic modelling has been proposed as an alternative method, but is deterministic in nature. We have developed a modelling structure that uses Monte Carlo simulation to introduce random variation into the parameters of these models, in order to simulate the behaviour of a population with interindividual variation.

We have applied this approach to a mathematical model describing the deposition, retention and clearance in the lung of a poorly soluble dust of low toxicity (TiO2), and inflammation resulting from the presence of the dust. Population variation (either animal or human) was simulated by generating 1000 variable instances of the key parameter sets. Predictions of lung burden, lymph node burden and recruitment of the inflammatory cells PMN were found to be most strongly influenced by individual breathing rates, and variation was close to linear in all the important parameters.

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SUMMARY

INTRODUCTION

The IOM recently completed a two-part study for the HSE looking at the pulmonary effects of two low toxicity dusts, in which a physiologically based mechanistic model of inhaled particles in rats was developed and validated using experimental data (Cullen et al., 1999; Tran et al., 1999). A physiologically based model was developed which described mechanistically the behaviour of low-toxicity dust in the lung, including deposition, clearance, interstitialisation and transfer to the lymph nodes. For risk assessment, it can be beneficial to consider inter-individual variation in the target population. The present work sought to extend the use of the model by introducing stochastic variation into the key parameters and examining the resulting distributions in the predicted model outputs. Consideration of this variation was essential for a full risk assessment.

Aims

The specific objectives were to:

i. undertake a sensitivity analysis of the current low toxicity dust model to determine the important physiological parameters in relation to predicted dose and effects;

ii. simulate the effect of inter-animal differences in the important parameters, and validate using data from earlier experiments;

iii. extrapolate the model from the rat to the human, and incorporate what is known about parameter distributions among human populations;

iv. undertake a probabilistic risk assessment of early responses in the lung for given exposure scenarios using the human model and extrapolate to lower concentrations and longer times to predict safe airborne concentrations of the specific ‘low toxicity’ dusts.

METHODS

Introduction of stochastic variation

The inhalation model described by Tran et al. (1999) consists of nine linked compartments within the lung and fourteen parameters describing the rates of transfer between these compartments. Further parameters controlled the rate of dust deposition in the lung as a function of airborne concentration. Principal outputs from the model are predictions of lung and lymph node dust burden and number of neutrophils (PMN) in the lung, at various times following the start of exposure.

We selected a set of key transfer rate parameters and simulated inter-individual variation in these by a Monte Carlo method. The parameters concerned breathing rate, macrophage mediated clearance rate, threshold lung burden, transfer to lymph nodes, and PMN recruitment coefficient. They were assumed uncorrelated and were randomly generated from statistical probability distributions having desired means and variances. A total of 1000 instances were generated, each having a different set of randomly generated parameters. Several parameters were held constant.

The model was run for each set of input parameters, yielding a set of 1000 output predictions.
Sensitivity and Uncertainty Analyses
Standard regression methods were used to investigate the dependence of the output predictions on the input parameters, and their relative contributions to the range of variation in those outputs. Smoothing curves were used to allow non-linear relationships.

Risk assessment based on animals
The model was rerun for the 1000 randomly generated instances, with a range of input exposure concentrations. The output distributions generated were compared with desired levels of the predicted responses, and concentrations identified that would induce suitably low levels of these.

This type of risk assessment was carried out for two different outputs, the overload of lung clearance, and the milder effect of initiation of inflammation.

Extrapolation to risk assessment for humans
Using the same underlying model structure, predictions for humans were formed by replacing rat parameter values with equivalent human values. Some human parameter values were derived by standard allometric scaling of parameters based on relative body weights of the two species (Clewell, 1995). For others, parameters were available directly from human studies, e.g. deposition efficiency in relation to breathing rates.

From the distribution of the predicted outputs for humans, a probabilistic risk assessment was undertaken. Again, predictions were rerun under differing exposure assumptions, and the output distribution of PMN numbers compared against the desired level for the initiation of inflammation (2% by number).

RESULTS

Sensitivity analysis
The 1000 sets of values for the model outputs showed considerable association between the lung and lymph node mass burdens of dust, and predicted PMN numbers were strongly related to lung burden, but less so to lymph node burden.

For lung burden, the breathing rate parameter $br$ was by far the main predictor, explaining 96% of the total sum of squares. The parameters for lung threshold burden, macrophage mediated clearance rate, and lymphatic transfer rate (respectively, $s_{\frac{1}{2}}$, $cl$, and $e$) explained about another 1% each. The PMN recruitment coefficient $Rec$ added nothing to the equation.

For the lymph node dust burden $br$ explained 79.3% of the total variation; $s_{\frac{1}{2}}$ and $e$ explained an additional 9%, and 8%, but $cl$ was a much poorer predictor, explaining about 0.4%, while $Rec$ again contributed nothing.

For PMN, the breathing rate $br$ was again the most important predictor (66% of total sum of squares), followed by the recruitment rate, $Rec$ (22%). Much smaller contributions came from $s_{\frac{1}{2}}$ (4%) and $cl$ (0.3%), and $e$ added nothing, leaving a residual standard deviation of 0.72.

For all three outputs, allowing non-linear fits improved the model only slightly.

Risk assessment based on animals - overload
One of the parameters allowed to vary between individuals was the threshold lung burden for overload, $s_{\frac{1}{2}}$. We defined the exposure concentration that would bring the individual animal's lung burden to this level as the Overload Threshold Exposure Concentration (OTEC). We then estimated the concentration that would be the threshold for only 5% of the population, i.e. would protect 95% of the population from overload. The OTEC was estimated at 8.7 mg.m$^{-3}$. 
Risk assessment based on animals - inflammation
Using the same technique of comparing outputs from different input concentrations, we sought to identify, as an NOAEL, the input concentration at which fewer than 5% of animals would show inflammation defined as PMN present at 2% of total cells. We estimated an NOAEL in the region of 4 mg.m\(^{-3}\), the current OES for human exposure.

Risk assessment for humans - inflammation
The predictions of PMN numbers from human parameter values were again compared across a range of input exposure concentrations. We retained the definition of inflammation as beginning when PMN constituted 2% of the total cells in the lung.

This critical exposure level was found to be 1.3 mg.m\(^{-3}\). Therefore, 1.3 mg.m\(^{-3}\) is suggested as an NOAEL for humans.

BENEFITS AND LIMITATIONS
The present work has extended our earlier deterministic model for the behaviour in the lung of inhaled particles of low toxicity, by allowing stochastic variation in the rate parameters, which in turn induces variation in the outputs. This allows us to simulate a population of individuals, and to include terms for inter-individual variation. This in turn allows investigation of the distribution of the modelled outputs, and determination of how to constrain the inputs so that the extremes of the output distribution fall below defined or acceptable limits. This approach has obvious applications in the setting of health-based occupational or environmental standards, and we have shown examples of exposure levels for TiO\(_2\) that should avoid clearance overload and inflammation in the majority (here 95%) of an exposed human population. Deriving limit values in this way substitutes modelling for the large-scale animal experimentation that would be necessary for traditional approaches, which is a benefit entirely in line with modern requirements to minimise the use of animals in experiments. We believe that this approach can be applied to other poorly soluble dusts encountered in the workplace.

Sensitivity and Uncertainty Analyses relating the output to the input variation has demonstrated that the model is relatively well-behaved, in that output responses are nearly linear in the variable inputs. This gives additional grounds for optimism that the model will be useful for other dusts.

These extensions necessarily introduce a number of new assumptions in the modelling process. The plausibility of the results rests directly on the plausibility of all the assumptions made about the model structure and the appropriate values of the rate parameters. However, explicit statement of all the assumptions allows informed discussion of these issues. It is not possible to estimate all of the parameters from a single data set, but many or all can be derived from basic principles or from experimental results in the literature. The dynamic model provides a framework for combining and integrating such observations from a wide variety of sources.
1. INTRODUCTION

1.1 BACKGROUND

Physiologically based mechanistic models are likely to play an increasing role in assessing the risks to human health of exposure to chemicals. These models describe the relationship between an external exposure and the internal dose to various organs. Used in conjunction with information on a toxic effect, these models allow a more precise derivation of dose-response relationships that are central to risk assessments. Mechanistic models also provide a more structured framework for extrapolation from the results in lab animals to predicted results in humans. Development of models that, in addition, predict the toxic effects resulting from internal dose, should contribute to a reduction in the use of lab animals.

Physiologically based pharmacokinetic (PBPK) models have been employed by some institutions and authorities in quantitative risk assessments of a variety of chemicals, notably in relation to cancer endpoints (Bailer and Dankovic, 1997). Physiologically based models have also been derived to describe the internal dose due to inhaled fibres and particles (Tran et al., 1995; 1997). These models are similar to the more traditional PBPK models in that they describe the transfer of particles among a series of inter-linked physiological compartments after deposition in the lung. However, little attention has been paid to dealing with population variation in the parameters of these models, which is necessary to explain the observed variability in animal data, or to the extrapolation of these models to predict for human populations.

The IOM recently completed a two-part study for the HSE looking at the pulmonary effects of so-called ‘low toxicity’ dusts, in this case TiO₂ and BaSO₄ (Cullen et al., 1999; Tran et al., 1999). One output of this study was the development of a physiologically based model which described mechanistically the processes involved after inhalation of low toxicity dusts by rats, e.g. deposition, clearance, interstitialisation, and transfer of particles to the lymph nodes. The model also described the early responses to inhaled dust, in particular the occurrence of ‘overload’, i.e. the impairment of clearance and recruitment of inflammatory cells into the lung. By incorporating the surface area dose of inhaled particles, the model could predict the effects of, for example, ultrafine dusts. After using data from inhalation experiments with rats for validation and calibration, the model was used to extrapolate to safe, or ‘no effect’, levels without recourse to further animal experimentation. Despite tightly controlled experiments, a large degree of inter-animal variation was observed in doses (e.g. lung burden) and effects (e.g. cell counts) that was not explained by the model for the ‘average’ rat. Attempts were made to adjust extrapolated safe levels to take account of this unexplained variation, but a full uncertainty analysis of the effects of parameter variability on model output variation was beyond the scope of that study.

This inhalation model for ‘low toxicity’ dusts proved effective in extrapolation to other exposure scenarios, thus reducing the need for animal experimentation. As such, it is a potentially useful tool in the setting of occupational standards. It will also form the basis for future models, extended to include the effects of inhaled soluble particles and more toxic substances such as silica. Enhancing this basic mechanistic model, to incorporate the effects of inter-individual variation in parameters, and extrapolating the model to human populations would be important contributions towards the use of such mechanistic models for assessing probabilistic risks to humans from inhaled particles and fibres.

Also, the model relates the observed level of the selected biomarkers to the eventual toxicological outcomes, taking account of their respective population variation. Potentially, the model could help to distinguish whether any observed level of a biomarker is within the normal range for an individual, or is indicative of toxicity.
1.2 OBJECTIVES

The overall aim of this study is to enhance the predictive capability of the particle inhalation model of Tran et al. (1999) and improve probabilistic risk assessment, by converting the model to human prediction and incorporating the effect of inter-individual variation in physiological parameters.

The specific objectives of the study were:

i. to undertake a full sensitivity analysis of the current low toxicity dust model to determine the important physiological parameters in relation to predicted dose and effects;

ii. to simulate the effect of inter-animal differences in the important parameters using what is known about population parameter variation and validate using observed data from earlier experiments;

iii. to extrapolate the model from the rat to the human, and incorporate what is known about parameter distributions among human populations;

iv. to undertake a probabilistic risk assessment of early responses in the lung for given exposure scenarios using the human model and extrapolate to predict safe airborne concentrations of the specific ‘low toxicity’ dusts.

1.3 METHODS

The methods used to achieve each of the above objectives are described below.

1.3.1 Sensitivity analysis

Methods of sensitivity analysis have been developed in recent years that allow the effect of each of a number of input parameters on output variation to be quantified. For example, powerful variance-based methods can quantify the influence of multiple input parameters, jointly and independently, on model output variance using a series of simulated computer experiments (e.g. Saltelli et al., 2000). Sensitivity analyses can also be used to check for structure within the model that may be redundant and hence indicate simplifications to the model.

The inhalation model described by Tran et al. (1999) consists of nine linked compartments within the lung, and fourteen parameters to describe transport of particles between these compartments. Further parameters are used to describe the deposition process. As a dynamic model, it predicts, for any given exposure pattern, the time course of lung and lymph node burdens and inflammatory cells.

Sensitivity analyses were carried out on this model to highlight the important input parameters for later attention.

1.3.2 Simulation

Most of the parameters for the current model were determined from currently accepted values in the literature (Tran et al., 1999), the remainder being estimated from observed data. Literature sources were searched for information on inter-animal differences in parameter values and, where this was not possible, parameter distributions were derived subjectively based on plausible ranges.

The deposited dose of dust in the lung is an important influence on subsequent lung burdens and responses. The deposition fraction of inhaled dust is commonly modelled as a function of the aerodynamic diameter of the dust particles, and uncertainties both in this function and the known diameter distribution of the dusts were taken into account in simulations. Differences in breathing
rates among rats, and fluctuations in actual exposure concentrations are sources of input variation that were also incorporated.

Monte Carlo simulations (Saltelli et al, 2000) were used to incorporate input parameter variation and to build up distributions of output variation through time for comparison with observed experimental data for lung burden and cell counts. Where possible, this included correlation between related parameters rather than assume all input parameters act independently. No new experimentation was undertaken for this study. Instead, full use was made of data from rat inhalation studies carried out at the IOM using TiO₂ (Cullen et al, 1999; Jones et al, 1988) in an attempt to capture all sources of variation in these experiments.

1.3.3 Extrapolation to humans

One of the benefits of a physiologically based mechanistic model, is that it provides a framework for extrapolation of animal data to humans. Using the same underlying model structure, predictions for humans are formed by replacing rat parameter values with equivalent human values. A starting point is to use standard allometric scaling of parameters based on relative body weights of the two species (Clewell, 1995). One example, however, where better quality data is available from human studies is for deposition efficiency in relation to breathing rates, which allows the effect of variations in deposited dose for given exposure scenarios to be explored. The power of simulation is the ability to test the model under several different ‘what-if’ scenarios, which were necessary for those parameters for which little human information is available.

1.3.4 Risk assessment

Using a series of candidate models for inhaled particles in humans, a probabilistic risk assessment of inhaled low toxicity dusts was carried out. This incorporated information on the effect of parameter variability on the probability of the early responses associated with the overload phenomenon under various exposure scenarios. An end product was the extrapolation to predict the maximum airborne concentration to which groups of individuals may be continuously exposed in an occupational setting with negligible risk of the onset of early pulmonary responses. In addition, the risk to health of continuous exposure to the current occupational exposure standard for low toxicity dusts (4 mg.m⁻³ respirable) was explored using the human inhaled particles model incorporating inter-individual parameter variation.

An important aspect of this risk assessment was the development of methods of presentation of the results, which stem from explicit assumptions on both parameter and model uncertainty, to help policy makers make informed decisions based on quantification of risks.

1.3.5 Benefits

This project seeks to add value to a previous HSE-funded project that developed a rat model for inhaled ‘low toxicity’ dusts. The developments should help to improve the practice of risk assessment of inhaled particles in human populations. The methodology, once validated using the specific hazard of ‘low toxicity’ dusts, will be applicable to further models that will take account of added complexities due to soluble particles, man-made and natural fibres, and more toxic particles such as silica.
2. BACKGROUND

2.1 MATHEMATICAL MODELS OF THE RETENTION AND CLEARANCE OF PARTICLES FROM THE LUNG

The earlier mathematical models of retention/clearance (e.g. Vincent *et al.*, 1990; Jones *et al.*, 1988; Yu *et al.*, 1990) described lung clearance of insoluble particles during chronic exposure in terms of clearance to the tracheobronchial region, transfer to lymph nodes and sequestration within the alveolar region.

Mathematically, the deposition and clearance process is a dynamic system, which can be described as a series of compartments. For example, in the model in this report, $X_1$ represents the quantity of free particles on the alveolar surface. Generally, the change in the particle burden in compartment $i$, $dX_i/dt$, is described by equations of the form:

$$\frac{dX_i}{dt} = D + I_{ij} - O_{ik}$$  \hspace{1cm} (2.1)

where $D$ = input from outside the system to compartment $i$,

$I_{ij}$ = input to compartment $i$ from compartment $j$,

$O_{ik}$ = output from compartment $i$ to compartment $k$.

Equation (2.1) is called the ‘mass balance’ equation (because, over a set of compartments, mass is preserved).

If the rate of transfer of particles from compartment $j$ to compartment $i$ is assumed to be directly proportional to the mass of particles resident in compartment $j$, i.e.:

$$I_{ij} = k_{ij} X_j$$ \hspace{1cm} (2.2)

then Equation (2.2) is called the ‘mass action’ type and $k_{ij}$ the ‘transfer rate’ is the fraction per unit time.

For multiple inputs and outputs Equation (2.1) can be generalised as:

$$\frac{dX_i}{dt} = D + \sum_{j=1}^{m} I_{ij} - \sum_{k=1}^{n} O_{ik} \quad i = 1, \ldots, l$$ \hspace{1cm} (2.3)

where $m$ is the number of compartments which output to compartment $i$, $n$ is the number of compartments which receive output from compartment $i$ and $l$ is the total number of compartments which make up the system.

A system of equations such as Equation (2.3) can represent the dynamics of the retention and clearance of particles/fibres in the alveolar region of the lung (Vincent *et al.*, 1987; Yu *et al.*, 1988; Strom *et al.*, 1988; Stöber *et al.*, 1989, 1990a,b; Katsnelson *et al.*, 1992).
2.2 STRUCTURE OF THE MATHEMATICAL MODEL

The model is defined by a set of differential equations, which describe the rates at which the quantities of particles in the various compartments are assumed to change. Below we describe these compartments, the scientific assumptions about the translocations between them, and the rate parameters governing these processes.

2.2.1 Compartments of the model

Our mathematical model describes the progress over time of the retention of particles and the alveolar macrophage (AM)-mediated clearance process in the pulmonary region, together with the particle redistribution and the overload phenomena. Figure 2.1 shows the nine conceptual compartments describing the location of inhaled particles, plus the main translocation routes between them, including AM-mediated clearance.

Figure 2.1
Schema of the compartments (X₁ to X₉) and the transfer rates between compartments.

In Figure 2.1, inhaled particles in the respirable range can reach the alveolar region of the lung, where they come into contact with epithelial cells. The mass (mg) of free particles on the alveolar surface is represented by compartment X₁. As the result of this contact, these particles are readily transferred into the interstitium (compartment X₂ represents the amount of free...
particles in the interstitium). However, the particle-epithelial cells contact also generates chemotactic signals that attract AMs to the site of particle deposition. The ensuing phagocytosis by AMs endeavours to clear the alveolar surface of particles (and thus prevent interstitialisation). Subsequently, the ingested particles are removed by migrating AMs to the mucociliary escalator (Compartment X\textsubscript{2} represents the amount of particles inside mobile, active AMs). However, these cells have a finite lifespan. AMs eventually decay and become inactive (Compartment X\textsubscript{5} represents the amount of particles inside decayed AMs) and release their particle load onto the alveolar surface for re-phagocytosis by other, more effective, AMs. Free particles which cross the alveolar epithelium into the interstitium may encounter interstitial macrophages (IMs) and the same events, as described above, are repeated (compartment X\textsubscript{6} represents the amount of particles inside mobile IMs and X\textsubscript{7} represents the particle amount inside decayed IMs). However, from the interstitium, some particles (both free and inside IMs) are removed to the lymph nodes (represented by compartment X\textsubscript{9}).

As the particle-epithelial cells contact progresses, AMs become increasingly retained in the alveolar region where they phagocytose until they become overloaded. As overloaded AMs decay, this load becomes increasingly difficult to redistribute to more effective AMs (i.e. the macrophages that ingest this particle load will, in turn, become overloaded). Gradually, a ‘sequestration’ pool of particles emerges, consisting of particles in overloaded AMs. This is represented by compartment X\textsubscript{4}. Similarly, interstitial granulomas are assumed to be derived from overloaded IMs. The amount of particles sequestered in granulomas is represented by compartment X\textsubscript{8} in the model. Table 2.1 gives a summary description of each of the compartments.

### Table 2.1
The compartments in the model representing the location of particles and the level of inflammation

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Location of particles</th>
</tr>
</thead>
<tbody>
<tr>
<td>(X_1)</td>
<td>free on alveolar surface</td>
</tr>
<tr>
<td>(X_2)</td>
<td>successfully phagocytosed by alveolar macrophages</td>
</tr>
<tr>
<td>(X_3)</td>
<td>in inactive alveolar macrophages, can be released for re-phagocytosis</td>
</tr>
<tr>
<td>(X_4)</td>
<td>sequestered in overloaded, immobile alveolar macrophages</td>
</tr>
<tr>
<td>(X_5)</td>
<td>free in interstitium</td>
</tr>
<tr>
<td>(X_6)</td>
<td>successfully phagocytosed by interstitial macrophages</td>
</tr>
<tr>
<td>(X_7)</td>
<td>attached to inactive interstitial macrophages, can be re-released for phagocytosis</td>
</tr>
<tr>
<td>(X_8)</td>
<td>interstitial granuloma</td>
</tr>
<tr>
<td>(X_9)</td>
<td>thoracic lymph nodes</td>
</tr>
</tbody>
</table>

PMN recruitment

| PMN | Number of PMN cells in the alveolar region |
2.3 MATHEMATICAL FORMULATION OF THE MODEL

2.3.1 The mathematical description of the normal (non-overload) retention and clearance of particles

i. On the alveolar surface

The rate of change of the mass of free particles (in mg.day\(^{-1}\)) consists primarily of the deposition of particles from the aerosol, the phagocytosis by alveolar macrophages and the interstitialisation of these particles and secondarily of the release of particles from macrophages which reach the end of their lifecycle:

\[
\frac{dX_1}{dt} = D - r_A X_1 - i X_1 + \delta_A X_3
\]  

(2.4a)

where \(X_1\) is the mass (mg) of free particles remaining on the alveolar surface;

\(D\) is the dose rate of particles deposited on the alveolar surface (mg.day\(^{-1}\)), calculated from Equation (2.4b);

\(r_A\) is the rate of phagocytosis by alveolar macrophages (day\(^{-1}\));

\(i\) is the rate of interstitialisation (day\(^{-1}\));

\(X_3\) is the mass (mg) of particles in macrophages in the inactive phase of their lifecycle;

and

\(\delta_A\) is the death rate for inactive macrophages.

The deposited dose rate \(D\) of deposited particles (in mg.day\(^{-1}\)) is calculated as:

\[
D = \text{Concentration} \times \text{Ventilation rate} \times \text{Daily Exposure period} \times \text{Alveolar deposition fraction} \times (5/7) \times (6/100)
\]

(2.4b)

where "Concentration" is the aerosol concentration (mg.m\(^{-3}\));

"Ventilation rate" is the breathing ventilation rate of the rat (l.minute\(^{-1}\));

"Daily Exposure period" is the duration of each daily exposure (hr.day\(^{-1}\));

"Alveolar deposition fraction" is the fraction of the inhaled particles of a given size deposited in the alveolar region and

\((5/7)\) converts the concentration for a five-days-per-week inhalation pattern into the equivalent average concentration for the 7-days week;

\((6/100)\) converts the units of the breathing rate to match the time and volume units of the concentration and exposure period.

The alveolar deposition fraction, used in Equation (2.4b), was derived in two ways: (i) from the assumption that inhaled particles are of the (Mass Median Aerodynamic Diameter) MMAD
size, and also (ii) from the measured particle size distribution, and using experimental data on the alveolar deposition efficiency for particle inhaled (Raabe et al., 1988).

The transfer rates \(D, r_A, i, \delta, \text{etc.}\) in these equations are shown in Figure 2.1 next to their translocation routes. The coefficients \(i, \delta, \text{etc.}\) are approximately constant when the lung burden is low, but at higher lung burden the macrophage mediated clearance becomes impaired and they become functions of the alveolar particle surface area, \(s_{alv}\); the form of this dependence is described later. This assumes that the dependence is on the sum of particles which are available to the AMs, i.e. dependence on \(s_{alv} = s.(X_1 + X_2 + X_3 + X_4)\), where \(s\) is particle specific surface area (in unit of area per unit of mass).

Equation 2.4a, with these coefficients written as functions of \(s_{alv}\), becomes:

\[
\frac{dX_1}{dt} = D - r_A X_1 - i(s_{alv}) X_1 + \delta_A(s_{alv}) X_3
\]  

Particles that have been phagocytosed by macrophages will subsequently either be removed from the alveolar region by way of macrophage migration and the mucociliary escalator, or be released onto the alveolar surface upon the necrosis of AMs. So the rate of change of the mass of phagocytosed particles in active AMs (i.e. \(X_2\)) is:

\[
\frac{dX_2}{dt} = r_A X_1 - c(l(s_{alv}) X_2 - \rho_A X_2
\]  

where \(c(l)\) is the AM-mediated clearance rate (day\(^{-1}\)), \(r_A\) is the phagocytosis rate (day\(^{-1}\)) and \(\rho_A\) is the transfer rate (day\(^{-1}\)) from active AMs to inactive AMs. When this clearance is unaffected by overload, \(c(l)\) is estimated to be 0.015 day\(^{-1}\) (Stöber et al., 1989). When clearance is affected by overload, then the dependence of \(c(l)\) on \(s_{alv}\) is described by Equation 2.13. The phagocytosis rate \(r_A\) is assumed to be independent of the particle surface area as AMs are assumed to be locally mobile in the alveolar region and able to phagocytose particles. Also, \(\rho_A\) is assumed to be unaffected by the particle surface area.

The mass of particles inside inactive AMs, \(X_3\), is described by:

\[
\frac{dX_3}{dt} = \rho_A X_2 - \delta_A(s_{alv}) X_3 - \phi(s_{alv}) X_3
\]  

where \(\delta_A\) is the release rate of particles from inactive AMs back to the alveolar surface and \(N\) is the rate of transfer into the alveolar sequestration compartment. Note that for a certain choice of \(\delta_A\) and \(\phi\), \(\delta_A + \phi = \text{constant}\).

Equations (2.4) to (2.6) describe the dynamics of translocation of particles on the alveolar surface when the lung defences are not overloaded. The fourth compartment on the alveolar surface, \(X_4\), becomes involved once the lung becomes overloaded with particles. The rate of change of the amount of particles in the alveolar sequestration compartment (\(X_4\)), representing the mass of particles trapped inside overloaded macrophages, is:

\[
\frac{dX_4}{dt} = \phi(s_{alv}) X_3
\]
ii. **In the interstitium**

Once particles are interstitialised, they will be phagocytosed readily by interstitial macrophages (IM). Interstitialised particles which escape phagocytosis, together with the particles phagocytosed by IMs may eventually be removed to the lymph nodes. Let $X_5$ be the mass of free particles which are interstitialised, then:

$$\frac{dX_5}{dt} = i(s_{alv})X_4 - e(s_{inst})X_5 - r_I X_5 + \delta_I(s_{inst})X_7$$ \hspace{1cm} (2.8)

where $e$ is the removal rate (day$^{-1}$) of particles to the lymph nodes;

$r_I$ and $\delta_I$, respectively the rates of phagocytosis by macrophages and release from inactive macrophages, are assumed to have the same value for IMs as for AMs;

and $s_{inst}$ is the interstitial burden in unit of surface area, i.e. $s_{inst} = s.(X_5 + X_6 + X_7 + X_8)$.

Equation (2.8) for IMs is comparable to Equation (2.4c) for AMs: the first term on the right hand side of equation (2.8) is the transfer from alveolar surface (instead of deposition in Equation (2.4c)), the second and third terms include $X_5$ instead of $X_1$, and the last term includes $X_7$ instead of $X_3$. Similarly, the mass of particles phagocytosed by IMs is:

$$\frac{dX_6}{dt} = r_I (s_{inst}) X_5 - e(s_{inst}) X_6 - \rho_I X_6$$ \hspace{1cm} (2.9)

where the removal rate to lymph nodes ($e$) is assumed to be the same for IMs as for interstitialised free particles.

The mass of particles trapped in interstitial granulomas is described by:

$$\frac{dX_7}{dt} = \rho_I X_6 - \delta(I_{s_{inst}})X_7 - \upsilon(I_{s_{inst}})X_7$$ \hspace{1cm} (2.10)

where the transfer rate of particles from active IMs to inactive IMs ($\rho_I$) and the release rate from inactive IMs ($\delta_I$) are also assumed to have the same dependence on the relevant burden (interstitial or alveolar particle surface area) and also the same non-overload values as for AMs; $\upsilon$ is the rate (day$^{-1}$) of interstitial granuloma formation which occurs when the IM defence of the interstitium becomes impaired.

The conditions relating to the transfer of particles to interstitial granuloma ($X_8$) are linked with overload and therefore are described in the section on overload (later). However, the mass of particles trapped in interstitial granulomas is described by:

$$\frac{dX_8}{dt} = \upsilon(s_{inst}) X_7$$ \hspace{1cm} (2.11)
iii. At the lymphatic level

The mass of particles accumulated in the mediastinal lymph nodes is the sum of the transfer from free interstitialised particles \((X_5)\) and particles in IMs \((X_6)\):

\[
\frac{dX_9}{dt} = e(X_5 + X_6)
\]  

(2.12)

2.3.2 Mathematical description of overload

As described earlier, the impairment of pulmonary clearance during exposure due to overload correlated with the increase in the rate of recruitment of PMNs. The PMN level, in turn, correlated with particle surface area. This impairment of clearance can be described mathematically as a function, \(\theta\), of alveolar particle burden (in terms of mass or surface area) which varies between 0 and 1. As \(\theta\) is a multiplier of the rate parameters, these parameters are fully functioning when \(\theta \approx 1\).

Mathematical expressions were developed to describe this progressive impairment. Similar equations were used in other models (e.g. Yu et al., 1988; Stöber et al., 1989; Tran et al., 1997). Note that all these functional forms are essentially chosen for practical reasons (i.e. they integrate well with the models in which they form a part). For example, Tran et al., 1997 used an exponential decay form

\[
\theta(m_{alv}) = e^{-\lambda(m_{alv} - m_{crit})^{\beta}} \quad \text{for } m_{alv} > m_{crit}
\]

\[
\theta(m_{alv}) = 1 \quad \text{for } m_{alv} \leq m_{crit}
\]

where \(m_{alv}\) is the particle mass in the alveolar region, \(m_{crit}\) is the critical mass from which impairment begins to manifest. \(\lambda\) and \(\beta\) are parameters controlling the rate and the form of decay. This function has two limitations. First, the parameters of this function cannot be related to some tangible entity, such as mass or surface area. So, it is difficult to judge the plausibility of different values which \((\lambda\) and \(\beta)\) give a good fit with data. Finally, there is a deterministic boundary at \(m_{crit}\) below which there is no impairment – i.e. the original was too abrupt. While there is some evidence that this might be the case (Muhle et al., 1990), in reality, impairment would likely progress continuously. Thus, a new functional form for \(\theta\) in terms of alveolar surface burden, \(s_{alv}\), is introduced:

\[
\theta(s_{alv}) = 1 - \frac{1}{1 + \left(\frac{s_{1/2}}{s_{alv}}\right)^{\beta}}
\]  

(2.13)
This functional form is similar to that used by Yu and Rappaport (1997) to describe retardation of clearance of insoluble dust. The function is dependent on two parameters, namely $s_{\frac{1}{2}}$ and $\beta$. The former, $s_{\frac{1}{2}}$, represents the level of particle surface area such that the impairment is half of its original value while the latter, $\beta$, controls the steepness of the impairment. Figure 2.2 shows the behaviour of $\theta$, for two different sets of values for $\beta$ and $s_{\frac{1}{2}}$ over a range of values of $s_{adv}$. One advantage this function has over the earlier functions from the literature is that one of its parameters, $s_{\frac{1}{2}}$, is readily interpretable and will be useful in the comparison of the effects of different dusts on their retention and clearance.

Since particle surface area affects clearance by mobile macrophages, we assume here that the clearance rate is modified as:

$$cl(s_{adv}) = \theta(s_{adv}) cl$$

where $cl$, on the right hand side of Equation (2.14), is the time-independent rate for low lung burdens. Thus, as the particle burden on the alveolar surface (in terms of surface area) increases, mobile macrophages are increasingly retained on the alveolar surface, as described by Equation 2.14. During this phase, particles released by inactive AMs upon death will be less likely to be removed by mobile AMs to the mucociliary escalator (i.e. the transfer rate $\delta_{A}$, back to the alveolar surface to be re-phagocytosed and then cleared by AMs, decreases with increasing alveolar lung burden). Instead, these particles are re-phagocytosed by retained AMs leading to transfer at a rate, $\phi$, the sequestration rate (day$^{-1}$), into an alveolar sequestration compartment ($X_4$). In this case, $\phi$ increases as impairment develops:
The impairment function for particle surface area between 0 to 750 cm$^2$ and two different sets of values for ($\beta$, $s_{1/2}$).

\[ \phi(s_{alv}) = (1 - \theta(s_{alv}))\phi \]  

(2.15)

and

\[ \delta_a(s_{alv}) = \theta(s_{alv})\delta_a \]  

(2.16)

For particles with large surface area, as inhalation progresses, there is increasing contact between these particles and epithelial cells, potentially causing damage to these cells. It is assumed that a damaged epithelium will allow greater access of particles into the interstitium. Therefore, the rate of particle interstitialisation increases concurrently with the progression of impairment. Mathematically, the rate of interstitialisation can be modelled as

\[ i(s_{alv}) = i_{normal} \theta(s_{alv}) + (1 - \theta(s_{alv})) \ i_{max} \]  

(2.17)

where $i_{normal}$ is the rate of interstitialisation under normal conditions and $i_{max}$ is the maximum rate of interstitialisation under complete impairment. So, according to this equation, initially $i(s_{alv}) = i_{normal}$ (≠ 0) because AM defence is not absolutely effective and there is always some interstitialisation taking place; once impairment starts, $i(s_{alv})$ increases from $i_{normal}$ towards $i_{max}$.

At the interstitial level, we assume that interstitial granuloma will be formed when the defence of the interstitium becomes impaired. There is, however, an absence of data regarding the particulate burden in the interstitium. Therefore, for the present, we are restricted to constructing the framework for this part of the model. This framework is presented to show
how the concepts can be included although the choice of values for the transfer rates will be limited to being plausible (but unsupported) and will also be chosen so as not to affect the predictions of quantities which can be tested by the existing data (for lymph node burdens).

For the current model, we assume that the impairment of clearance for IMs by dust loading has the same form of dependence on dust loading as for the AMs. We also assume that the impairment of motility follows the same dependence on the impairment function $\theta$ thus:

$$\nu(s_{\text{inst}}) = (1 - \theta(s_{\text{inst}})) \nu$$  \hspace{1cm} (2.18)

The differential equations (2.4 to 2.18), describing the kinetics of the retention and clearance of particles under normal circumstance (i.e. low exposure and non-impairment of AM defence mechanisms) and for the overload situation, constitute the current mathematical model. The model provides a quantitative, logical representation of the mechanisms of removal of particles from the lung.

The above equations describing the effect of particulate overload describe the process that results in a higher proportion of the lung burden entering the interstitium. The presence of more particles in the interstitium makes more particles available for transfer to the mediastinal lymph nodes. However, there does not appear to be a reason why a higher proportion of the interstitialised particles should be transferred to lymph nodes, so the coefficient for transfer from interstitium to mediastinal lymph nodes ($X_i$) remains constant.

### 2.3.3 Mathematical description of PMN recruitment

In this section, the original model is extended to describe the inflammatory recruitment of PMN cells. There is an association between the mean number of PMNs in the BAL fluid and the mean lymph node burden, expressed as surface area (Tran et al., 1999). Since particles found in the lymph nodes were originally interstitialised, the net rate of PMN recruitment is assumed to be proportional to the rate of particle interstitialisation (expressed as surface area) and a PMN removal rate which is attributed to normal lifecycle of this type of cell. Thus,

$$\frac{dPMN}{dt} = \text{Rec}.i(s_{\text{salv}}).s.X_i - \text{Rem}.PMN$$  \hspace{1cm} (2.19)

where PMN represents the number of PMNs ($x10^6$) in the BAL fluid. Rec is the number of PMNs recruited per unit of dust interstitialised (as surface area). Rem is the removal rate of PMNs (day$^{-1}$). The specific particle surface area is $s$ and $X_i$ is the mass of free particles on the alveolar surface.

### 2.3.4 Summary of model parameters

The translocations between the compartments of the model are expressed by transfer rates (labelled in Figure 2.1 and defined in Table 2.2). These rates determine the fraction of mass of particles per unit time which are translocated from one compartment to another (e.g. $r$, the phagocytosis rate of macrophages, represents the fraction of particles transferred from $X_1$ the compartment of free particles on the alveolar surface, to $X_2$ the compartment of successfully phagocytosed particles). In addition to the transfer rates, there are parameters belonging to the impairment function (e.g. $\beta$ and $s_{\text{half}}$ in Equation (2.13)) and those belonging to the deposited dose $D$ (e.g. breathing rate, deposition fraction, etc…).
**Table 2.2**
The parameters of the mathematical model

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Symbol</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEPOSITION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deposited dose rate, function of breathing rate, deposition efficiency and exposure concentration</td>
<td>$D$</td>
<td>mg.day$^{-1}$</td>
</tr>
<tr>
<td><strong>KINETICS IN MACROPHAGES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phagocytosis rate by AMs or IMs †</td>
<td>$r_A, r_I^*$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>AM-mediated clearance of particles</td>
<td>$cI$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Transfer rate of particles from active to inactive AMs or IMs †</td>
<td>$\rho_A, \rho_I$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Release rate of particles back to the alveolar surface or interstitium for re-phagocytosis †</td>
<td>$\delta_A, \delta_I$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td><strong>KINETICS OF PARTICLES</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal interstitisation rate of free particles</td>
<td>$i_{normal}$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Maximum interstitisation rate of free particles</td>
<td>$i_{max}$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Removal rate of particles to the lymph nodes</td>
<td>$e$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td><strong>OVERLOAD AND SEQUESTRATION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar sequestration rate</td>
<td>$\phi$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Rate of formation of interstitial granuloma</td>
<td>$\nu$</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td><strong>PMN RECRUITMENT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN recruitment rate</td>
<td>$Rec$</td>
<td>$N^o$ of cells recruited per unit of particle surface area burden</td>
</tr>
<tr>
<td>PMN removal rate</td>
<td>$Rem$</td>
<td>day$^{-1}$</td>
</tr>
</tbody>
</table>

†The subscripts A and I indicate that the coefficients apply, respectively, to the alveolar and interstitial macrophages.
2.4 MODEL PARAMETERS

2.4.1 Parameter values

Table 2.3 shows the values of the parameters used in the calculation of the deposited dose $D$ (Equation 2.4b). Table 2.4 shows the values of the parameters drawn from previous studies, to be used in the model. The parameters include the phagocytosis rate which is based on experimental evidence (described by Stöber et al. (1989, 1990a,b, 1991)) indicating that phagocytosis usually takes place within 2 to 6 hours and so, following Stöber, we use the 6 hour estimate. This is equivalent to a phagocytosis rate of approximately $1/6 = 0.166 \text{ hour}^{-1}$, or equivalently $4 \text{ day}^{-1}$ when expressed in the same units as the other rates in Table 2.2. The macrophage mediated clearance rate has been estimated as ranging from $0.01 \text{ day}^{-1}$ to approximately $0.02 \text{ day}^{-1}$, with the value of $0.015 \text{ day}^{-1}$ being commonly applicable (e.g. Stöber et al., 1989). Estimates of the time scales for the macrophage normal life cycle (also based on the evidence presented by Stöber et al. (1990a,b, 1991)) were used to estimate the rate of transfer from active to inactive macrophages ($\rho$) and for release from inactive macrophages either for re-phagocytosis (transfer rate $\delta$) or after overload to become trapped in a succession of overloaded macrophages (transfer rate $\phi$). For example, if the time scale for the active phase of the life cycle is $T_a$ days, then a population of macrophages in kinetic equilibrium would have a fraction of $1/T_a$ of the active macrophages pass from active to inactive phase each day; so $\rho = 1/T_a$. Similarly, there would be a rate of death and release of particles from inactive macrophages $\delta$ equal to $1/T_i$, where $T_i$ is the time scale of the inactive phase. Both $T_a$ and $T_i$ have been originally estimated by Stöber et al. (1989, 1990) and values of $T_a (=28 \text{ days})$ and $T_i (= 7 \text{ days})$ from their studies, corresponding to a reasonable estimate of AM total life-cycle of 35 days (Van Oud Alblas et al., 1986), was used in our model.

Table 2.3
Factors affecting the deposited dose

<table>
<thead>
<tr>
<th>Factor</th>
<th>Value</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breathing rate ($l$/min)</td>
<td>0.1-0.3</td>
<td>Stöber et al., (1994); Yu et al., (1994).</td>
</tr>
<tr>
<td>Target Concentrations (mg.m$^{-3}$)</td>
<td>50 mg m$^{-3}$</td>
<td>TiO$_2$</td>
</tr>
<tr>
<td>Exposure regimen</td>
<td>7 hrs/day, 5 days/week</td>
<td></td>
</tr>
<tr>
<td>Correction factor (to treat exposure over 5 days as continuous over the week)</td>
<td>$\frac{5}{7} = 0.714$</td>
<td>(Also used by Morrow, 1988)</td>
</tr>
<tr>
<td>TiO$_2$ deposition fraction</td>
<td>0.07</td>
<td>Original estimates derived from $in \ vivo$ data used in this study, and consistent with values from Raabe et al 1977 and 1988.</td>
</tr>
</tbody>
</table>
Table 2.4
The a priori fixed model parameters

<table>
<thead>
<tr>
<th>PARAMETERS</th>
<th>Symbol</th>
<th>Value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DEPOSITION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deposited dose rate, function of breathing rate,</td>
<td>$D$</td>
<td>see</td>
<td>mg.day$^{-1}$</td>
</tr>
<tr>
<td>deposition efficiency and exposure concentration</td>
<td></td>
<td>Table 2.3</td>
<td></td>
</tr>
<tr>
<td><strong>KINETICS IN MACROPHAGES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phagocytosis rate by AMs or IMs †</td>
<td>$r_A, r_I$</td>
<td>4</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>AM-mediated clearance of particles</td>
<td>$c_l$</td>
<td>0.015</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Transfer rate of particles from active to inactive AMs or IMs †</td>
<td>$\rho_A, \rho_I$</td>
<td>0.036</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Release rate of particles back to the alveolar surface or interstitial for re-phagocytosis †</td>
<td>$\delta_A, \delta_I$</td>
<td>0.14</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Transfer from overloaded IM to granuloma</td>
<td>$\nu$</td>
<td>0.14</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td><strong>KINETICS OF PARTICLES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interstitialisation of free particles, normal rate</td>
<td>$i_{normal}$</td>
<td>0.03</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Interstitialisation of free particles, maximum rate</td>
<td>$i_{max}$</td>
<td>1.8</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Removal rate of particles to the lymph nodes</td>
<td>$e$</td>
<td>0.1</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td><strong>OVERLOAD AND SEQUESTRATION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar sequestration rate</td>
<td>$\phi$</td>
<td>0.14</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>Rate of formation of interstitial granuloma</td>
<td>$\nu$</td>
<td>0.14</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td><strong>IMPAIRMENT FUNCTION</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overload threshold</td>
<td>$m_{\frac{1}{2}}$</td>
<td>5.8</td>
<td>mg</td>
</tr>
<tr>
<td>Overload constant</td>
<td>$\beta$</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td><strong>PMN RECRUITMENT</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMN recruitment rate</td>
<td>$Rec$</td>
<td>0.025</td>
<td>N$^0$ of cells per unit of s.a. burden</td>
</tr>
<tr>
<td>PMN removal rate</td>
<td>$Rem$</td>
<td>0.01</td>
<td>day$^{-1}$</td>
</tr>
</tbody>
</table>

†The subscripts A and I indicate that the coefficients apply, respectively, to the alveolar and interstitial macrophages.
The rate of particulate deposition into the lung was estimated from the volume inhaled, the aerosol concentration and the alveolar deposition fraction. The values estimated for the breathing rate and alveolar deposition fraction, and the plausible range, are listed in Table 2.3. A wide range of values for the breathing rate is plausible as various studies have used markedly different estimates, as shown in the first row of Table 2.3. The alveolar deposition fraction has been measured in rats, as a function of the particle aerodynamic diameter, by Raabe et al. (1988) giving estimates of 7% for the TiO$_2$ particles. Table 2.4 shows all the parameters of the model. These parameters were kept constant during all the model simulations.

2.5 PARAMETERS SELECTED FOR SENSITIVITY ANALYSIS

Each of the parameters in Table 2.5 were chosen to vary because they are likely to play a significant part in the dose-response relationship. For example $br$, the breathing rate, influences the amount of dust deposited on the alveolar surface. The AM-mediated clearance of particles, as represented by the parameter $cl$, is another important factor controlling the alveolar dose (lung burden). The surface area threshold, $s_{1/2}$, determines when the clearance becomes impaired and is consequently of importance to our investigation. The translocation rate to the lymph nodes, determining the lymph node burden, is likely to be different between individuals, as reflected in the variation seen in the data. Similarly, for the PMN recruitment rate. The rest of the parameters were kept constant because they affect only the internal compartments, (e.g. the interstitium) and not the total dose (lung burden).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$br$</td>
<td>Breathing rate</td>
</tr>
<tr>
<td>$cl$</td>
<td>Clearance rate</td>
</tr>
<tr>
<td>$s_{1/2}$</td>
<td>Surface area threshold</td>
</tr>
<tr>
<td>$e$</td>
<td>Rate of translocation to lymph node</td>
</tr>
<tr>
<td>$Rec$</td>
<td>PMN recruitment rate</td>
</tr>
</tbody>
</table>
3. SENSITIVITY ANALYSIS

3.1 INTRODUCTION

For complex numerical models, Sensitivity Analysis (SA) is the study of how the outputs of a given model depend on the information that is fed into it. The model is defined as a system of equations, which involve a range of variables, parameters and input factors (which we may combine under the label "inputs"). If the behaviour of the system is complex, then its response to changes in the inputs may be far from linear, and may be unpredictable without actually running the model for different combinations of input. Often, the effects of changes in one input will depend on the value of another input, and a complete investigation will require running the model with many combinations of inputs.

Sensitivity Analysis is often linked with Uncertainty Analysis (UA), which also looks at variation in model outputs. Model input factors may be subject to uncertainty from a range of sources, including errors of measurement, absence of information, and even poor or partial understanding of the driving forces and mechanisms. If we run a series of models with varying inputs, we can gain understanding of the variations to be expected in the process being modelled; and if the variation in the inputs is typical of the variation in some target population, we gain insight into the likely range of variation across that population, in the outputs being modelled. A simple example is how variations in metabolic rate between individuals in a population would affect the active amount of a drug in a target organ at particular times, and how that active amount would vary given the same input dose.

Rather than considering SA and UA as alternative approaches, they can be seen as two aspects of attempting to understand the behaviour of our model. SA is usually concerned with sensitivity in terms of local variation around some average set of inputs, while UA implies a more global overview, concerned with the whole range of variation or uncertainty that might need to be considered. Perhaps confusingly, partitioning the total variation into contributions from different inputs is considered a topic in SA. However, both must proceed by running the model with varying sets of inputs and analysing how the variation in outputs relates to these. The ideal situation is where both can be addressed from the same set of model runs.

We have applied a Monte Carlo simulation technique in the present work. In this approach, the expected variation in selected inputs is described by statistical distributions, and we draw random values from these distributions to represent variable input values. We may refer to a set of inputs generated this way as defining an instance of the model, and each randomly drawn set of inputs represents a new instance. The statistical distributions may be independent; alternatively, some or all of the inputs may be correlated, and require to be drawn from a multivariate distribution. The choice depends on context, and the aim is to simulate the characteristics of variation in a particular target population.

3.2 VARIATION IN THE INPUTS AND SIMULATIONS

The model of Fig 2.1 depends on multiple parameters, as described in Chapter 2. A number of these are considered well-determined, and their values are given in Table 2.4. We are concerned with the effects of variation in the selected parameters (Section 2.5), listed in Table 3.1. This Table also shows the statistical distributions chosen to represent the uncertainty in the parameter values. For uniform distributions, we show the range of possible values, over which the values have uniform probability. The uniform distribution is used for the breathing rate only, simulating a population where any individual may be at rest or active. Several parameters are modelled as having log-normal distributions (in which the logarithm of the value has the normal or Gaussian distribution), and for these we show the geometric mean.
along with the values bracketing 95% and 99.73% (2 and 3 geometric standard deviations each side).

The distributions in Table 3.1 are independent, reflecting a view that these inputs are not correlated in the target experimental animal population. We drew 1000 samples from each distribution, creating 1000 sets of inputs, each defining an instance of the model. Each instance was run to simulate the inhalation experiment with TiO$_2$ whereby rats were exposed at 50 mg.m$^{-3}$ for 103 days. This yielded output predictions of lung and lymph node mass burdens of dust, and numbers of PMNs present in the lung, at the end of the exposure period.

### 3.3 THE REGRESSION TECHNIQUES

The 1000 instances generated by the Monte Carlo simulation produced a data set of which each unit consisted of the five input parameters plus the three predicted outputs from that instance of the model. The task of Sensitivity and Uncertainty Analyses was to relate the variation in the outputs to the variation in the inputs, and regression analysis is a useful tool for such problems. Traditional regression techniques have focussed mostly on only linear models, but as we have noted there is no guarantee that linearity will be a good approximation in linear models, or that the inputs will not interact (have joint effects). We have fitted regressions relaxing the linearity assumption by fitting Generalized Additive Models (Hastie and Tibshirani, 1990), which fit smooth curves whose shapes are dictated by the data and not by parametric assumptions. In the present work, we contrast linear regression models, with 1 degree of freedom (d.f.) for each input, with GAM models with three d.f. for each. The additional two d.f. are allotted to the curvature of the smoothed model.

Initially, each of the outputs was analysed independently. In order to further understand the behaviour of the model, some additional models were fitted where, for example, the predicted lung burden became in turn a predictor for lymph node burden.

### 3.4 RESULTS

Table 3.1 summarises the actual distributions of the 1000 random samples of the five model parameters. Comparison with Table 3.2 shows that these distributions are consistent with the specifications for the sampling.

Figure 3.1 shows the distributions of the outputs from the instances of the model defined by the 1000 random samples. These are summarised as box plots, in which the central box extends from the 25th to the 75th percentile; a line within the box marks the position of the median; the whiskers outside the box extend to the 10th and 90th percentiles; and the 10% lowest and 10% highest values are plotted as individual triangular symbols. The predicted lung burdens had a broadly symmetrical distribution, with a median value of nearly 15 mg, 10th and 90th percentiles of about 7 and 21 mg, and no values below 4 mg or above 25 mg. Lymph node burdens were much lower, approaching zero in some cases, never exceeding 7.2 mg. Predicted numbers of PMN were somewhat less symmetrical.

Figure 3.2 shows pair-wise scatter plots summarising the relationships between the model outputs. Considerable association can be seen between the lung and lymph node mass burdens of dust. Predicted PMN numbers were also strongly related to lung burden, but less so to lymph node burden.

Linear and GAM regression models were fitted to the lung mass burden using stepwise selection procedures, which had a standard deviation of 5.08. The four log-normal parameters were offered to the regression both on the log scale and on the parameter scale; in general, the log scale gave a slightly better linear fit, and all the results shown here use the logs. The
breathing rate parameter \( br \) was by far the first choice of predictor, explaining \( 24729.5 \) (96%) of the total sum of squares of \( 25742.3 \). The parameters \( s_{3/4}, e \) and \( cl \) explained about another 1% each, and \( Rec \) added nothing to the equation. The residual variation not explained by the five-parameter linear regression had a standard deviation of 0.53. Inclusion of the additional two d.f. for a non-linear fit in each term improved the overall fit slightly, particularly in the fit to \( e \), and the residual standard deviation was reduced to 0.47.

The lymph node dust burden had a raw standard deviation of 1.81. Of a total sum of squares of 3267.4, \( br \) explained 2590.2 (79.3%). \( s_{3/4} \) and \( e \) explained an additional 9%, and 8%, but \( cl \) was a much poorer predictor, explaining about 0.4%, while \( Rec \) again contributed nothing. The residual variation had a standard deviation of 0.35. Again, inclusion of non-linear terms made a small difference, again mostly to the fit with \( e \), and the resulting standard deviation was 0.28.

For PMN, the raw standard deviation was 2.56. In the linear regression, the breathing rate \( br \) was again the most important predictor (66% of total sum of squares), followed by the recruitment rate, \( Rec \) (22%). Much smaller contributions came from \( s_{3/4} \) (4%) and \( cl \) (0.3%), and \( e \) added nothing, leaving a residual standard deviation of 0.72. Inclusion of the extra d.f. for non-linearity improved these values only slightly, leaving a residual standard deviation of 0.68.
Table 3.1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Assumed distribution</th>
<th>Min</th>
<th>Max</th>
<th>Geom Mean</th>
<th>Geom Std Devn</th>
<th>95% range</th>
<th>99.7% range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
</tr>
<tr>
<td>br Breathing rate</td>
<td>Uniform distribution</td>
<td>0.1</td>
<td>0.3</td>
<td>(0.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>cl Clearance</td>
<td>Log-normal</td>
<td>0</td>
<td>∞</td>
<td>0.015</td>
<td>1.189</td>
<td>0.011</td>
<td>0.021</td>
</tr>
<tr>
<td>s½ Surface area threshold</td>
<td>Log-normal</td>
<td>0</td>
<td>∞</td>
<td>387</td>
<td>1.246</td>
<td>251</td>
<td>596</td>
</tr>
<tr>
<td>e Translocation to lymph node</td>
<td>Log-normal</td>
<td>0</td>
<td>∞</td>
<td>0.10</td>
<td>2.154</td>
<td>0.022</td>
<td>0.450</td>
</tr>
<tr>
<td>Rec PMN recruitment</td>
<td>Log-normal</td>
<td>0</td>
<td>∞</td>
<td>0.025</td>
<td>1.291</td>
<td>0.015</td>
<td>0.041</td>
</tr>
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</table>

Table 3.2

<table>
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<th>Parameter</th>
<th>Assumed distribution</th>
<th>Geom Mean</th>
<th>Geom Std Devn</th>
<th>95% range</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Geom Mean</td>
<td>Geom Std Devn</td>
<td>95% range</td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lower</td>
<td>Upper</td>
<td></td>
</tr>
<tr>
<td>br Breathing rate</td>
<td>Uniform distribution</td>
<td>(0.202)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.100</td>
</tr>
<tr>
<td>cl Clearance</td>
<td>Log-normal</td>
<td>0.015</td>
<td>1.186</td>
<td>0.011</td>
<td>0.021</td>
<td>0.009</td>
</tr>
<tr>
<td>s½ Surface area threshold</td>
<td>Log-normal</td>
<td>387</td>
<td>1.246</td>
<td>251</td>
<td>596</td>
<td>205</td>
</tr>
<tr>
<td>e Translocation to lymph node</td>
<td>Log-normal</td>
<td>0.10</td>
<td>2.190</td>
<td>0.022</td>
<td>0.450</td>
<td>0.006</td>
</tr>
<tr>
<td>Rec PMN recruitment</td>
<td>Log-normal</td>
<td>0.025</td>
<td>1.297</td>
<td>0.015</td>
<td>0.041</td>
<td>0.009</td>
</tr>
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</table>
Table 3.3
Contribution of each of five parameters to linear and smooth GAM regressions for three model outputs: based on Monte Carlo simulation of 1000 instances of model

<table>
<thead>
<tr>
<th>Parameter included</th>
<th>Lung burden</th>
<th>Linear regression</th>
<th></th>
<th>GAM smooth regression</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sum of squares</td>
<td>% of total</td>
<td>Sum of squares</td>
<td>% of total</td>
</tr>
<tr>
<td>$br$</td>
<td></td>
<td>24729.5</td>
<td>96.1</td>
<td>24750.6</td>
<td>96.1</td>
</tr>
<tr>
<td>$s_{1/2}$</td>
<td></td>
<td>278.5</td>
<td>1.1</td>
<td>289.6</td>
<td>1.1</td>
</tr>
<tr>
<td>$e$</td>
<td></td>
<td>242.2</td>
<td>0.9</td>
<td>271.6</td>
<td>1.1</td>
</tr>
<tr>
<td>$cl$</td>
<td></td>
<td>210.2</td>
<td>0.8</td>
<td>207.9</td>
<td>0.8</td>
</tr>
<tr>
<td>$Rec$</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
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<tr>
<td>Residual</td>
<td></td>
<td>281.9</td>
<td>1.1</td>
<td>221.6</td>
<td>0.9</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>25742.3</td>
<td>100.0</td>
<td>25742.3</td>
<td>100.0</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter included</th>
<th>Lymph node burden</th>
<th>Linear regression</th>
<th></th>
<th>GAM smooth regression</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sum of squares</td>
<td>% of total</td>
<td>Sum of squares</td>
<td>% of total</td>
</tr>
<tr>
<td>$br$</td>
<td></td>
<td>2590.3</td>
<td>79.3</td>
<td>2598.7</td>
<td>79.5</td>
</tr>
<tr>
<td>$s_{1/2}$</td>
<td></td>
<td>296.1</td>
<td>9.1</td>
<td>299.4</td>
<td>9.2</td>
</tr>
<tr>
<td>$e$</td>
<td></td>
<td>248.5</td>
<td>7.6</td>
<td>278.7</td>
<td>8.5</td>
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<tr>
<td>$cl$</td>
<td></td>
<td>14.1</td>
<td>0.4</td>
<td>15.7</td>
<td>0.5</td>
</tr>
<tr>
<td>$Rec$</td>
<td></td>
<td>0.0</td>
<td>0.0</td>
<td>0.2</td>
<td>0.0</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>118.4</td>
<td>3.6</td>
<td>74.8</td>
<td>2.3</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>3267.4</td>
<td>100.0</td>
<td>3267.4</td>
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</table>

<table>
<thead>
<tr>
<th>Parameter included</th>
<th>PMN</th>
<th>Linear regression</th>
<th></th>
<th>GAM smooth regression</th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Sum of squares</td>
<td>% of total</td>
<td>Sum of squares</td>
<td>% of total</td>
</tr>
<tr>
<td>$br$</td>
<td></td>
<td>4304.3</td>
<td>65.7</td>
<td>4333.9</td>
<td>66.1</td>
</tr>
<tr>
<td>$Rec$</td>
<td></td>
<td>1443.8</td>
<td>22.0</td>
<td>1474.0</td>
<td>22.5</td>
</tr>
<tr>
<td>$s_{1/2}$</td>
<td></td>
<td>261.4</td>
<td>4.0</td>
<td>264.1</td>
<td>4.0</td>
</tr>
<tr>
<td>$cl$</td>
<td></td>
<td>20.5</td>
<td>0.3</td>
<td>20.1</td>
<td>0.3</td>
</tr>
<tr>
<td>$e$</td>
<td></td>
<td>0.4</td>
<td>0.0</td>
<td>1.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Residual</td>
<td></td>
<td>521.9</td>
<td>8.0</td>
<td>458.3</td>
<td>7.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td></td>
<td>6552.3</td>
<td>100.0</td>
<td>6552.3</td>
<td>100.0</td>
</tr>
</tbody>
</table>
Figure 3.1
Box plots showing distributions of three separate output variables from model with Monte-Carlo simulated inputs, 1000 instances.
Figure 3.2
Scatter plots showing bivariate distributions of three separate output variables from model with Monte-Carlo simulated inputs, 1000 instances.
4. ANIMAL BASED PROBABILISTIC RISK ASSESSMENT

4.1 DEFINITION OF NOAEL

An important feature of our model is that it incorporates the phenomenon of overload, taken to mean the point at which normal clearance mechanisms begin to be impaired. With inter-individual variation, overload may be induced under different exposure regimes, but by incorporating plausible assumptions about the nature of that variation, we can examine more realistically the variation in predicted outputs with varying concentration. This in turn permits determination of the concentration level at which most or all of the individuals would not develop overload.

In our specific formulation, overload is controlled by the overload threshold parameter $s_{0\%}$. We have therefore sought to estimate the level of exposure concentration at which the lung burden in the majority of individuals would not exceed $s_{0\%}$. We designate this as the Overload Threshold Exposure Concentration (OTEC).

However, it would not be appropriate to treat this concentration as a No Observable Adverse Effect Level (NOAEL), because even without overload the lung may respond to exposure with inflammation, represented by the recruitment of PMN cells. We therefore propose that any NOAEL for exposure to non-toxic dust should take account of the need to avoid inflammation.

Illing (1991) discussed the difficulty of defining an NOAEL in general, and cited various definitions of NOAEL including the definition given by the International Programme on Chemical Safety/Joint FAO/WHO committee on Food Additives (IPCS/JECFA, 1987):

‘The greatest concentration or amount of an agent, found by study or observation, that causes no detectable, usually adverse, alteration of morphology, functional capacity, growth or lifespan of the target’.

Currently, the US EPA define NOAEL as: “An exposure level at which there are no statistically or biologically significant increases in the frequency or severity of adverse effects between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered as adverse, or as precursors to adverse effects. In an experiment with several NOAEIs, the regulatory focus is primarily on the highest one, leading to the common usage of the term NOAEL as the highest exposure without adverse effect.” (http://www.epa.gov/OCEPAterms/nterms.html).

In this study, the effect in which we are interested is the beginning of inflammation, which we choose to define as the detection of PMNs in the BAL fluids at 2% or more of total cells. This choice of definition is in part arbitrary: to date there is no consensus in the research community as to how high the PMN level would have to be in order to constitute a toxicologically important event. However, the normal level of PMN in the lung is well below 1% of the cell population, so the presence of 2% of PMN is a reliable indicator of a response. We follow conventional usage in defining as an NOAEL the level of exposure that will avoid this adverse but mild effect in 95% of the target population.

4.2 INVESTIGATION INTO INTER-ANIMAL VARIATION

The data from the assays in our previous experimental studies (Cullen et al 1999, Tran et al 1999) have shown considerable variations between animals within a time point. These variations may be due to the difference between animals in some of the key parameters, such as
the breathing rate, the AM-mediated clearance rate, translocation rate to the lymph nodes and the inflammatory reaction to dust.

The sensitivity analysis of the model parameters (Chapter 3) demonstrated which parameters are most influential (i.e. which parameters are such that a small change in their value would lead to a large change in the model outputs). The next step was to compare the variation in the predicted outputs with that observed in the same variables in actual animal experiments.

Figure 4.1 shows the range of variation (median plus 5th and 95th percentiles) in the three predicted results, lung burden, lymph node burden and the PMN cell count, along with the experimental data, which are plotted as mean and 2 standard deviations. It is clear that the predicted variation is consistent with the observed, at least in order of magnitude.

Figure 4.1.
The build up of (i) lung burden; (ii) lymph node burden and (iii) PMN number, with data from (Tran et al, 2000); the error bars are 2 x standard deviation. In each graph, the solid curve represents the model simulation using the central parameter sets while the 95th and 5th percentile of the variation are represented by the upper and lower dotted curves respectively.
4.3 DETERMINATION OF EXPOSURE CONCENTRATION AT OVERLOAD THRESHOLD BY SIMULATION

Our simulations and model predictions assume individual variation in parameters including the overload threshold, and it follows that the level of exposure required to reach this threshold must vary between individuals. However, the required level, which we define as the Overload Threshold Exposure Concentration (OTEC), can be estimated for each individual by interpolation. We simulated chronic (2-year) inhalation experiments, for a range of aerosol respirable dust concentrations starting from 70 mg.m\(^{-3}\) and moving to a concentration level such that the final lung burden was just below the individual’s overload threshold (\(s_{\text{OT}}\)).

Figure 4.2 shows the distribution of the estimated OTECs together with the 5\(^{\text{th}}\) and 50\(^{\text{th}}\) percentiles and the current occupational exposure standard (OES) for low toxicity dust, 4 mg.m\(^{-3}\).

By definition, the 5\(^{\text{th}}\) percentile OTEC is the level of concentration such that, in the chronic inhalation experiment, 95 percent of the population will avoid overload. From the distribution of OTEC this is calculated as 8.7 mg.m\(^{-3}\) for TiO\(_2\). This is higher than the current OES of 4 mg.m\(^{-3}\), at which essentially the entire rat population will avoid overload.

![Figure 4.2.](image)

The OTEC distribution together with the current OES (4 mg.m\(^{-3}\)), 5\(^{\text{th}}\) percentile (8.7 mg.m\(^{-3}\)) and 50\(^{\text{th}}\) percentile (18 mg.m\(^{-3}\)).

The concentration of 8.7 mg.m\(^{-3}\) represents a level at which 95% of our hypothetical rat population would avoid overload as defined by \(S_{\text{OT}}\).
4.4 ESTIMATION OF NOAEL FOR INFLAMMATION

At the OTEC, estimated at a concentration of 8.7 mg.m$^{-3}$, 95% of our theoretical rat population will live their lives without developing overload. However, exposure at that level, if sustained over a whole lifespan, is likely to produce at least some inflammation (e.g. in smokers, Rom et al., 1987). The next step was to determine exposure and the relationship between inflammation as evidenced by PMN recruitment. We therefore reran the model predictions, using the same 1000 generated instances of parameter values, but again with a lower range of input airborne concentrations.

Figure 4.3 shows, for the output variables representing PMN numbers and lymph node burden, the time course of the mean and 95th percentile of the distribution of the predictions. At 5 mg.m$^{-3}$ for TiO$_2$, the steady-state 95th percentile for PMN is over $0.1\times10^6$, approximately 2% of total cell numbers (not shown). Further simulations for the exposure to the current control limit, 4 mg.m$^{-3}$, show that the 95th percentiles of the responses at this concentration level are all well below this level. Thus, the current control limit for TiO$_2$ is below the NOAEL for rats exposed at the current exposure regimen (Table 2.3) for 2 years.

Figure 4.3.

NOAEL: Time-related PMN numbers for the exposure concentrations of 5 mg.m$^{-3}$ (top graph) and 4 mg.m$^{-3}$ (lower graph), with the dotted line representing the 95th percentile of animals and the solid line representing the average response.
The simulations of animal exposures have shown:

(i) For the TiO$_2$ (specific surface area = 6.68 m$^2$/g and MMAD = 2.1 µm gsd) the model estimated that at 8.7 mg.m$^{-3}$ (of respirable dust measured by Casella MRE113A) 95% of the rat population would avoid overload as defined by s$_{5/3}$; (i.e. 8.7 mg.m$^{-3}$ is the 5$^{th}$ percentile OPTEC);

(ii) With a no-observed-adverse-effect-level (NOAEL) defined as requiring a low level of PMN no greater than 2% following a 2-year inhalation exposure, a NOAEL of 5 mg.m$^{-3}$ was obtained;

(iii) Furthermore, examination of the 95$^{th}$ percentile of the populations’ simulated responses at the current control limit for respirable low toxicity dust (4 mg.m$^{-3}$) indicated that for TiO$_2$ exposure, 95% of rats would be well below the above definition of a NOAEL.

The simulations have thus produced estimates of safe levels of exposure for lifetime (2 year) exposure in rats, by extrapolation from data from relatively short term experiments with only limited numbers of animals, whereas experimental determination of NOAEL levels would have required large numbers of animals.
5. **EXTRAPOLATION TO HUMANS**

5.1 **MODEL PARAMETERS TO BE EXTRAPOLATED**

The extrapolation in Chapter 4 is one method for calculating a safe limit (NOAEL) from animal data, and those results serve as guidance for humans. In this chapter, we present an approach for extrapolating to human situations with the same dynamic model structure as described in Chapter 2, using human-specific parameters from (a) information available for humans where possible and/or (b) scaled parameter values obtained from animal data. The resulting human equivalent model is used to simulate the exposure-dose-response relationship in humans with a working life-time exposure to TiO$_2$ at various relevant airborne concentration levels.

The four main areas, in the exposure-dose-response relationships, that are expected to differ between rats and humans are: (i) Exposure concentration; (ii) Deposited Dose; (iii) Retention and Clearance and (iv) Cell Recruitment. Occupational human exposure is usually at lower airborne concentration and longer duration than the exposure in animal studies. The deposited dose is influenced by the ventilation rate and the deposition fraction. Both these parameters are dependent on the morphology of the lung and are expected to differ between species. Once deposited, particles are either retained or cleared; the retained particles are either interstitialised (parameters $i_{\text{normal}}, i_{\text{max}}$) and removed to the lymph nodes ($e$) or cleared by macrophages ($cl$). The retention and clearance of particles is known to vary between species (Bailey et al., 1985). The impairment of particle clearance following overload ($s_{\text{½}}$) is also known to be species dependent, with rats being more sensitive than humans (Nikula et al., 1997).

Table 5.1 lists the model parameters that were either estimated or scaled to humans. The remaining parameters were kept fixed at the values estimated from animal data.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Deposited Dose</th>
<th>Retention and Clearance</th>
<th>Cell Recruitment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration</td>
<td>Ventilation rate</td>
<td>Cl</td>
<td>Rec</td>
</tr>
<tr>
<td>$t_{\text{start}}$</td>
<td>Deposition fraction</td>
<td>$e$</td>
<td></td>
</tr>
<tr>
<td>$t_{\text{final}}$</td>
<td>$s_{\text{½}}$</td>
<td>$i_{\text{normal}}$</td>
<td></td>
</tr>
<tr>
<td>Daily exposure period</td>
<td>$i_{\text{max}}$</td>
<td>$i_{\text{normal}}$</td>
<td></td>
</tr>
</tbody>
</table>
5.2 METHOD FOR EXTRAPOLATION

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

(i) for Exposure (Concentration, \( t_{\text{start}}, t_{\text{final}} \))

We replaced the parameters for concentration and duration with the relevant human occupational equivalents (e.g. 4 mg.m\(^{-3}\) and working life time of 40 years).

(ii) for Deposited Dose (Ventilation rate, Deposition fraction)

We used data available from Hattis et al (2001) (see Table 4.2).

(iii) for Retention and Clearance (\( c_{l}, e, i_{\text{normal}}, i_{\text{max}} \))

We scaled parameters inversely with the ratio of pulmonary surface area to the power 0.25, in accord with the method of Ings (1991).

\[ c_{l_{\text{human}}} = c_{l_{\text{rat}}}, \text{ (rat pulmonary surface area } / \text{human pulmonary surface area})^{0.25}. \]

This produces an estimate of the lung clearance rate for humans which is consistent with other estimates of the rate for humans (Heid et al, 1988; Bailey et al, 1982; Miller et al, 2000).

(iv) for Threshold Burden (\( s_{0.5} \))

We expressed the critical lung burden in units of mg/g lung of rat then multiplied by human lung weight to get an absolute value for this parameter for humans (Morrow, 1988) then converted into surface area units using the specific surface area of the TiO\(_{2}\).

(v) for Cell Recruitment (Rec)

The recruitment of PMN and their removal are events which take place in relation to the particle dose. In this chapter the recruitment rate for PMNs is scaled inversely with the ratio of pulmonary surface area (rat/human).

(vi) for Parameter Distribution

The 1000 randomly generated parameter sets described in chapter 3 were converted from rat to human parameters. This approach was applied to all model parameters of Table 5.1, except for the ventilation rate, deposition fraction and clearance rate for which we have independent information from Hattis et al (2001). These parameters’ distribution characteristics are given in Table 5.2.

### Table 5.2.

Central value for Ventilation rate, Deposition fraction and Clearance rate and their distribution (Hattis et al, 2001).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Distribution</th>
<th>( \log_{10}(GSD) )</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ventilation rate</td>
<td>Log-normal</td>
<td>0.12</td>
<td>1.7 (m(^3)/hr)</td>
</tr>
<tr>
<td>Deposition fraction</td>
<td>Log-normal</td>
<td>0.30</td>
<td>0.092</td>
</tr>
<tr>
<td>Clearance (( c_{l} ))</td>
<td>Log-normal</td>
<td>0.21</td>
<td>0.0036 (day(^{-1}))</td>
</tr>
</tbody>
</table>
5.3 RESULTS

5.3.1 Results from parameter extrapolation

Table 5.3 shows the mean values for each of the parameters, for rats and for humans.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rat</th>
<th>Human</th>
</tr>
</thead>
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<tr>
<td><strong>EXPOSURE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concentration (mg.m⁻³)</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>( t_{\text{start}} ) (yr)</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>( t_{\text{end}} ) (yr)</td>
<td>2.00</td>
<td>45.0</td>
</tr>
<tr>
<td><strong>DEPOSITED DOSE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deposition fraction</td>
<td>0.06</td>
<td>0.32</td>
</tr>
<tr>
<td>Ventilation rate (m³/hr)</td>
<td>0.18</td>
<td>13.5</td>
</tr>
<tr>
<td>Hours exposed (hr/day)</td>
<td>7</td>
<td>8</td>
</tr>
<tr>
<td><strong>RETENTION AND CLEARANCE</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( c_l ) (day⁻¹) )</td>
<td>0.015</td>
<td>0.0036</td>
</tr>
<tr>
<td>( i_{\text{normal}} ) (day⁻¹)</td>
<td>0.03</td>
<td>0.0072</td>
</tr>
<tr>
<td>( i_{\text{max}} ) (day⁻¹)</td>
<td>1.8</td>
<td>0.4347</td>
</tr>
<tr>
<td>( e ) (day⁻¹)</td>
<td>0.1</td>
<td>0.0242</td>
</tr>
<tr>
<td>( m_{\frac{1}{2}} ) (mg)</td>
<td>5.8</td>
<td>4.05 x 10³</td>
</tr>
<tr>
<td><strong>CELL RECRUITMENT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R_{\text{e}} )</td>
<td>0.025</td>
<td>8.67 x 10⁻⁵</td>
</tr>
<tr>
<td><strong>LUNG PARAMETERS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung weight (gm)</td>
<td>1.43</td>
<td>1000</td>
</tr>
<tr>
<td>Lung surface area (cm²)</td>
<td>4865</td>
<td>1430000</td>
</tr>
</tbody>
</table>

The data available from Hattis et al (2001) were used to construct the distribution of values for the ventilation rate, deposition fraction and clearance rate in humans. The results are shown in Figure 5.1.
Figure 5.1.
The distribution for (i) ventilation rate; (ii) Deposition fraction; (iii) Clearance rate (data from Hattis et al, 2001).

5.3.2 Simulation Results

The human-scaled model was run for the 1000 human parameter sets, for a human population with a 45-year exposure at 4 mg.m$^{-3}$ of respirable dust (TiO$_2$), working on an 8 hour shift per day and 250 days per year. The results for the three main assays: lung burden, lymph node burden and number of PMN cells, are shown in Figure 5.2.

For each assay, the upper curves represent the 95$^{th}$, 90$^{th}$, 85$^{th}$ and 70$^{th}$ percentile of the variation. The two lowest curves for each assay, are generated using the central values in Table 5.2 and the 5$^{th}$ percentile. At a level of 4 mg.m$^{-3}$ (below the NOAEL level as derived for rats in Chapter 4), the extrapolated model predicted the occurrence of overload in approximately 30 percent of the human population. This is indicated in the retardation of clearance in the build up of lung burden for the 95$^{th}$, 90$^{th}$ and 85$^{th}$ percentile curves. The pattern of overload diminished at lower percentiles and from the 70$^{th}$ percentile, pulmonary clearance of dust is unimpaired.
Figure 5.2.
Simulation of the dose-response to TiO$_2$ in humans. (a) lung burden; (b) lymph node burden and (c) number of PMN cells. Each curve represents the 95$^{th}$, 90$^{th}$, 85$^{th}$ and 70$^{th}$ percentile of the variation in each assay. The two lowest curves for each assay, are generated using respectively the central value in Table 5.2 and the 5$^{th}$ percentiles.

Figure 5.3.
The dose-response at 1.3 mg.m$^{-3}$. (a) lung burden; (b) lymph node burden and (c) number of PMN cells. For each assay, the upper curve represents the 95$^{th}$ percentile of the variation; the two lowest curves are generated using respectively the central values in Table 5.2 and the 5$^{th}$ percentiles.
To find the level of airborne concentration such that 95 percent of the population can avoid overload, further extrapolations at lower exposure concentrations were needed. Simulations with stepwise decreases in concentration were made until concentration reached a level such that the 95 percent of the population does not develop overload within a life-time working exposure. This critical exposure level was found to be $1.3 \text{ mg.m}^{-3}$. Also, at this concentration, the predicted PMN level was low compared to the population of AM ($7 \times 10^9$ cells; Kuempel et al, 2001). Therefore, $1.3 \text{ mg.m}^{-3}$ is suggested as a NOAEL for humans.

Figure 5.2 shows the results of the further extrapolations: The upper curve represents the 95th percentile level for each assay. The central curve is obtained from the central values in Table 5.2 and the lower curve represents the fifth percentile.
6. DISCUSSION

6.1 MATHEMATICAL MODEL

In modelling complex biological processes, it is often possible to describe broad trends using simple regression or other statistical models. However, when it is desired to model the evolution of a process over time, and particularly when the status of the process at any time point influences its subsequent course, it is necessary to develop dynamic models based around differential equations. In either case, the model will be more plausible if based upon an understanding of the underlying biological mechanisms.

A model is a mathematical equation or system of equations that, given quantitative inputs, predicts certain outputs; and, given the same inputs, the output prediction will always be the same. In considering the relevance of model predictions to real-life situations involving populations of animals or of humans, there is an additional need to allow for the variation to be expected in any such population.

The present work has extended our earlier deterministic model by allowing stochastic variation in the parameters, which in turn induces variation in the outputs. Since the input variation is under our control and known, we can investigate the relationships between input and output variation. Such investigations are commonly labelled Sensitivity Analysis when the focus is on the effect of small variations in the inputs, and Uncertainty Analysis when we consider the entire range of variation. In the present work, we have used Monte Carlo simulation, with plausible assumptions for parameter variation, to generate a pseudo-sample of 1000 instances from an idealised population of rats. From this, we have been able to predict levels of exposure concentration of nuisance dusts such as TiO$_2$, which are likely to protect the majority of the population from clearance overload, or from the onset of inflammation. We have also been able to scale the results to an idealised human population, with the purpose of estimating an NOAEL for nuisance dusts. We believe that our approach to this problem is novel.

It is clear that these extensions to the basic process of dynamic modelling introduce a number of new assumptions, and that the plausibility of the results rests in a large part on the plausibility of the assumptions, in particular on the implied ranges of variability.

6.2 SENSITIVITY ANALYSIS

Good modelling practice requires that the modeller should provide an evaluation of the confidence in the model, assessing the uncertainties (i.e. uncertainties in the model structures, assumptions, and specifications) associated with the modelling process and with the outcome of the model itself. As part of this assessment, we applied SA analysis to the 1000 predicted model outputs generated by the Monte Carlo exercise. Using standard regression techniques, we investigated the effects on (i) the level of lung burden, (ii) lymph node burden and (iii) PMN number at the end of the exposure period (103 days) of changes in the input parameters. Findings included;

(a) For the variation in lung burden, the most influential parameters was that representing the rate of deposition into the lung, the breathing rate ($br$). Then three aspects of the clearance process had about equal impact on the variability in the results: the threshold lung burden ($s_{th}$), and the rates for translocation to the lymph node ($e$) and AM-mediated clearance ($cl$).
(b) For lymph node burden, \( br \) again was the parameter accounting for most of the variation (79%). Then parameters \( s_\parallel \) and \( e \) accounted for the next level (about 9% each). The lymph node burden variation is thus driven by the depositing lung burden, then to an approximately equal extent, by the threshold for overload and the rate of transfer to the lymph nodes.

(c) For PMN number, again the deposition parameter was the most important predictor, followed by the PMN recruitment rate \( Rec \). This is consistent with the assumption that expressed by the model that recruitment of PMN would be in proportion to the transfer of free particles from the alveolar surface to the interstitium. The overload threshold has a slight effect on the amount of free particulate, so under these assumptions it is reasonable to find that the sensitivity analysis shows that it is the third most significant of the parameters, but contributing much less than the first two.

Interpretation of all these findings was relatively straightforward, because most of the trend in each parameter was nearly linear. This is principally because the model is not strongly controlled by feedback, and its response to small changes is therefore approximately linear. The relative importance of the different input parameters was consistent with prior expectation on the basis of biological mechanisms.

6.3 PROBABILISTIC RISK ASSESSMENT

The introduction of stochastic variation to a dynamic but essentially deterministic model induces a probabilistic distribution in the predicted outputs. This can be used to relate risks of exceeding given output to input conditions, e.g. long-term exposure concentrations. This is an essential part of a risk assessment, and to the determination of limiting values such as NOAELs. We have applied this approach to two different possible outcome criteria, concerning the overloading and breakdown of the lung's natural clearance mechanisms, and the initiation of inflammation.

6.3.1 Overload of clearance: the OTEC

By assigning an appropriate distribution for each of the key parameters representing deposition, clearance, translocation and cellular response, we were able to reproduce the level of variation seen in the experimental data. The central model parameters and the distributions assigned to the key parameters were able to predict the time course of the lung and lymph node burdens and to mirror the observed inter-animal variations. Manipulation of the input exposure concentrations allowed us to estimate that a concentration of 8.7 mg.m\(^{-3}\) would be associated with overload in less than 5% of the animal population. We defined this as the Overload Threshold Exposure Concentration (OTEC). However, it is not clear that this concept has an immediate use in regulating exposure.

This is because a lifetime's exposure at the OTEC level may still induce a relatively high PMN level, so it cannot be considered to produce no observed adverse effect. Thus, further extrapolations to lower concentrations were required, to estimate a level at which inflammation would be largely avoided.

6.3.2 Inflammation: the NOAEL

We have found that, at the current OEL, 4 mg.m\(^{-3}\), the 95th percentile of the distribution of predicted PMN was well below 2% of the total cells; and in fact that an input concentration of
5mg.m\(^{-3}\) produced a 95\(^{th}\) percentile of almost exactly 2%. Therefore, we propose that a concentration of 4 mg.m\(^{-3}\) meets our current definition of NOAEL with a small margin of safety.

6.3.3 Extrapolation to humans

The current model was based on animal data. A human equivalent model was constructed by replacing some of the (animal based) parameters by their known human equivalent (e.g. the deposition fraction). For parameters with unknown human equivalents, appropriate allometric scaling methods were used for their estimation. Data available from literature (Hattis et al, 2001) regarding the variation in some key parameters in humans, such as the deposition fraction and clearance rate, have shown a much wider variation than the observed variation in animal data. This was no surprise as the rats are all from the same strain and the same supply.

A Monte Carlo simulation of the human based model, for a life-time exposure at 4 mg.m\(^{-3}\) suggested that only 70 percent of the population avoided overload. A concentration level such that 95 percent of the population can avoid overload was found to be 1.3 mg.m\(^{-3}\). At this level, the PMN number was negligible. Therefore, 1.3 mg.m\(^{-3}\) is proposed as an NOAEL for humans.

6.4 CONCLUDING REMARKS

In this study, we have introduced new methodologies to improve the quantitative risk assessment process for particle exposure. At the core of our approach was a mathematical model, describing the exposure-dose-response relationship for a poorly soluble particle, TiO\(_2\). This deterministic model was first tested for sensitivity with respect to key model parameters and was subsequently extended to incorporate inter-animal variation. The model was used in a probabilistic risk assessment, first based on animal data and then extrapolated to humans. This study has illustrated the necessary steps involved. Our approach can be revised in the light of new information from animal experiments and human data and applied to other poorly soluble dusts, such as carbon black.

The principal advantage of our approach is that, while risk assessments for humans almost always require extrapolation from animal risks, we have estimated these by modelling rather than by animal experimentation, in line with current ethical trends towards minimising experimentation on animal subjects.
7. ACKNOWLEDGEMENTS

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8. REFERENCES


