Interindividual and interoccasion variability of toxicokinetic parameters of uptake, exhalation, and metabolism of ethylene

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Abstract

A basic part in the risk assessment of potential carcinogens is the determination of toxicokinetic parameters. The partition of the xenobiotic in the body of experimental animals is a first step of the biochemical pathway of the formation of DNA adducts which might lead to the development of cancer.

The aim of extrapolation of toxicological data from experimental animals to the human organism requires a valid characterisation of the considered processes for the whole species, i. e. population parameters, moreover accounting for the variability within and between individuals.

This paper presents the results of an inhalation study with one of the basic petrochemical industrial compounds, ethylene (ethene). Two nonlinear four-stage hierarchical models for a repeated measurement design which are of different complexity are presented. The estimation of the individual and population parameters as well as of the covariance matrices is performed by an EM algorithm.

Key Words: ethylene, ethylene oxide, risk assessment, toxicokinetics, population parameters, two-compartment model, nonlinear hierarchical model, Bayes estimates, EM algorithm, repeated measurement

1. Introduction

A basic part in the risk assessment of potential carcinogens is the determination of toxicokinetic parameters. Most chemical carcinogens are transformed into a chemical active form, its metabolite, that is able to interact with cellular macromolecules such as DNA, RNA, and protein, and might finally lead to the development of cancer. The relationship between applied dose and tumor response is nonlinear (Bolt and Filser, 1984). This nonlinearity is supposed to be connected with the kinetic processes involved in the formation of DNA adducts (Hoel et al., 1983). Hence an important step to assess the risk of a xenobiotic is to investigate the kinetic processes of its uptake, metabolism, and elimination.

As the complete research depends on experiments with animals, a critical step is the extrapolation from the risk observed in the experimental animals to the risk associated with the human organism. The basis of such a species extrapolation are the so called PBPK-models (*physiologically-based pharmacokinetic models*) which take consideration of many strongly connected kinetic processes. These models require detailed information about physiological parameters, as well as information about the processes involved. The physiological parameters are supposed to be valid for a whole population. Determining kinetic population parameters, the variation between individual parameters which may also vary between repeated occasions and doses should be taken into account.

The present study has been designed to elucidate interindividual and interoccasion variabilities of toxicokinetic parameters relevant for the carcinogenicity of one of the basic petrochemical industrial compounds, ethylene (ethene) (Selinski et al., 1999).

Therefore two groups of inhalation experiments with male Sprague-Dawley rats were performed at the Institute of Occupational Physiology at the University of Dortmund. In the first group (group A) 10 rats were exposed 5 times each to a concentration of 100 ppm. In the second group (group B) another 10 animals were exposed to five different concentrations of 20, 50, 100, 200, and 500 ppm ethylene each (Quinke et al., 2000; Selinski, 2000; Selinski and Urfer, 1998). This paper refers only to models for the first experimental design. Estimates for the second group will be presented in a further paper.

Ethylene is an important industrial bulk chemical, which is also present in the environment. In mammalian organisms ethylene is partly transformed, by hepatic metabolising enzymes (cytochrome P-450) to ethylene oxide (Filser and Bolt, 1983). Ethylene oxide, also a physiological body constituent (Bolt, 1996, 1998; Bolt et al., 1997), is biologically reactive and thereby genotoxic (Kirkovski et al., 1998). The principles of the toxicokinetics of this transformation have been extensively studied (Filser and Bolt, 1984; Bolt et al., 1984), and estimates of the carcinogenic risk of ethylene based on its metabolic transformation to ethylene oxide were published (Bolt and Filser, 1984, 1987). As previous inhalation experiments with ethylene have indicated the metabolism may be well approximated by first order kinetics at concentrations below 800 ppm (*parts per million).* This approximation is used in the present study where the maximum concentration were about 500 ppm ethylene. At higher concentrations the metabolism of ethylene becomes more and more saturated (Bolt and Filser, 1987).

A two-compartment model is applied to describe the processes of uptake, exhalation, and metabolic elimination of ethylene. Two nonlinear four-stage hierarchical models of different complexity based on the approach of Racine-Poon and Smith (1990) are presented. The estimation of the individual and population parameters as well as of the covariance matrices is performed by an EM algorithm as proposed by Dempster et al. (1977).

2. Two-compartment model

The two-compartment model used by Filser (1992) for the characterisation of exposure to volatile xenobiotics describes uptake, endogenous production, excretion, and the metabolic elimination of the substance. The model is depicted as follows: a xenobiotic gas, in this case ethylene, enters the body and is exhaled. This process is described by introducing two compartments, the first, C_1, representing the environment outside the body, here the inhalation chamber of the exposition system, and the second compartment, C_2, the body itself. The volatile xenobiotic migrates from one compartment to the other through a theoretical interface. During this process, some portion of the xenobiotic within the organism, at any stage, is eliminated by metabolic processes, and another portion is again exhaled (cf. Fig. 1).

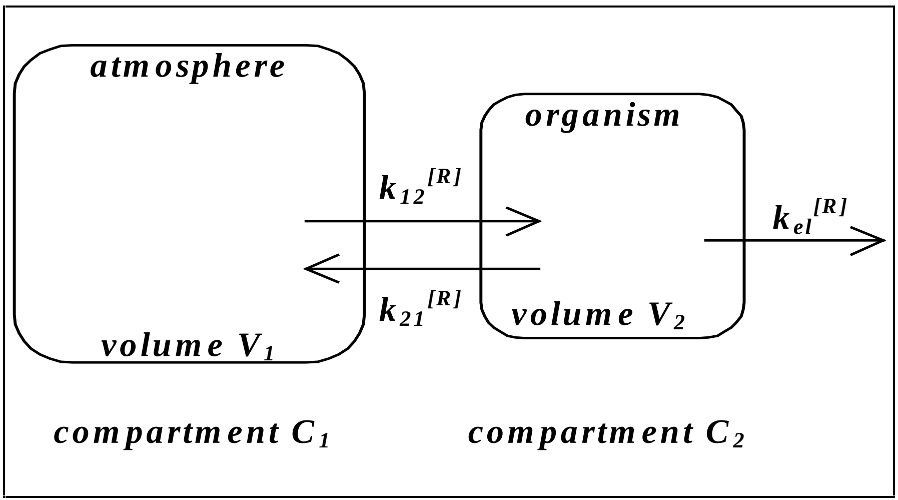

Figure 1. *Two-compartment block model in the case of metabolic turnover*

This paper concentrates on overall first order kinetic processes as preceding investigations have indicated that the initial concentrations from 20 to 500 ppm which we used here were below the point of saturation of ethylene at about 800 ppm. Thus, the processes may be well approximated by first order kinetics (Bolt and Filser, 1987). Moreover, Becka (1998) showed that first order kinetics may be used as approximations for nonlinear kinetic processes, e.g., Michaelis-Menten kinetics, if the observed maximum concentrations do not exceed the point of saturation.

Let $y_l(t)$, $l = 1, 2$, denote the concentration of a xenobiotic in compartment l at time t and let V_l describe the volume of the compartment. A preliminary assumption is that the compound, in this case ethylene, is metabolised within the body, and that there is no metabolism back to the parent ethylene, the latter being very likely on toxicological grounds.

In the case of overall first order kinetics, each partial process can be characterised by one rate or velocity constant k, that is $k_{12}^{[R]}$ for the uptake, $k_{21}^{[R]}$ for the exhalation, and $k_{el}^{[R]}$ for the metabolic elimination (cf. Fig. 1).

Thus, the concentration of ethylene in the first and in the second compartment, respectively, is given by (Becka et al., 1993; Urfer and Becka, 1996):

$$y_1(t) = y(0) \cdot \left\{ \frac{\left(k_{12}^{[R]} + \lambda_1\right)\exp\{\lambda_2 t\} - \left(k_{21}^{[R]} + \lambda_2\right)\exp\{\lambda_1 t\}}{(\lambda_1 - \lambda_2)} \right\}, \text{ and} \tag{1}$$

$$y_2(t) = y(0) \cdot \left\{ \frac{\left(k_{12}^{[R]} + \lambda_1\right)\left(k_{12}^{[R]} + \lambda_2\right)}{(\lambda_1 - \lambda_2)\alpha_2 k_{21}^{[R]}} \cdot \left[\exp\{\lambda_2 t\} - \exp\{\lambda_1 t\}\right] \right\}, \text{ respectively,} \tag{2}$$

where $\lambda_{1,2} = \frac{1}{2}\left\{-\left(k_{12}^{[R]} + k_{21}^{[R]} + k_{el}^{[R]}\right) \pm \sqrt{\left(k_{12}^{[R]} + k_{21}^{[R]} + k_{el}^{[R]}\right)^2 - 4k_{12}^{[R]}k_{el}^{[R]}}\right\}$ and $y(0)$ is the initial concentration in compartment 1.

In the practical application we have to take into account, that the individual organisms have different volumes which are also varying between repeated experimental occasions. In general, the kinetic parameters of the individuals are estimated first and then standardised to eliminate the effect of the volume (i.e., slightly different body weights of the rats). As we use the estimated parameters of the individuals for further calculations, we estimate the standardised kinetic parameters directly (Selinski et al., 1999; Selinski, 2000).

According to Filser (1992) the individual rates of uptake $k_{12}^{[R]}$, exhalation $k_{21}^{[R]}$ and metabolic elimination $k_{el}^{[R]}$ are equal to the respective rates k_{12}, k_{21} and k_{el} for a standard rat of 1000 ml times a fraction of a volume dependent factor $v_2 = \left(\frac{1000}{V_2}\right)$, where V_2 is the actual volume of the organism directly (Selinski et al., 1999; Selinski, 2000).

Substituting the real kinetic parameters in the (1) and (2) yields

$$f(\beta, t) = y_1(t) = y(0) \cdot \left\{\frac{\left(k_{12}v_2^{2/3} + \lambda_1\right)\exp\{\lambda_2 t\} - \left(k_{21}v_2^{1/3} + \lambda_2\right)\exp\{\lambda_1 t\}}{(\lambda_1 - \lambda_2)}\right\}, \tag{3}$$

and

$$y_2(t) = y(0) \cdot \left\{\frac{\left(k_{12}v_2^{2/3} + \lambda_1\right)\left(k_{12}v_2^{2/3} + \lambda_2\right)}{(\lambda_1 - \lambda_2)\alpha_2 k_{21}v_2^{1/3}} \cdot \left[\exp\{\lambda_2 t\} - \exp\{\lambda_1 t\}\right]\right\}, \tag{4}$$

where $\lambda_{1,2} = \frac{1}{2}\left\{-\left(k_{12}v_2^{2/3} + k_{21}v_2^{1/3} + k_{el}v_2\right) \pm \sqrt{\left(k_{12}v_2^{2/3} + k_{21}v_2^{1/3} + k_{el}v_2\right)^2 - 4k_{12}k_{el}v_2^{5/3}}\right\}$

and $\beta = (k_{12}, k_{21}, k_{el}, y(0))^T$ is the vector of the standardised kinetic parameters and the initial concentration $y(0)$.

3. Population models

3.1 Notation

The observed concentrations of ethylene in the atmosphere of the exposition system (compartment 1) are denoted by y_{ijk}, with

$i = 1, \ldots, I$ the number of the individual rat

$j = 1, \ldots, J$ the observations at time points t_j and

$k = 1, \ldots, K$ the number of the experiment.

Equal time points of measurement are only assumed to simplify the notation. The index k denotes the kth occasion of exposure to 100 ppm ethylene.

First of all, we presume that our observations y_{ijk} can be described by a nonlinear function $f(\beta_{ik}, t_j)$ and an error term ε_{ijk}:

$$y_{ijk} = f(\beta_{ik}, t_j) + \varepsilon_{ijk}, \quad i = 1, \ldots, I, j = 1, \ldots, J, k = 1, \ldots, K.$$

The function $f(\beta_{ik}, t_j)$ depends on the individual parameter vector β_{ik} and the time t. It denotes the expected concentration-time curve of the ith individual at the kth occasion. The parameter vector $\beta_{ik} = (k_{12ik}, k_{21ik}, k_{elik}, y_{ik}(0))^T = (\varphi_{ik}^T, y_{ik}(0))^T$, where $\varphi_{ik} = (k_{12ik}, k_{21ik}, k_{elik})^T$ represents the vector of the standardised kinetic parameters, differs from individual to individual and is of dimension $p = 4$.

Due to the way of application, the initial concentrations $y_{ik}(0)$ are not exactly known and have to be treated as parameters, although we are merely interested in the kinetic parameters.

Our main interest are not the individual responses to the experimental conditions but is focussed on a population mean process, which underlies the different individual processes. The individual kinetic parameter vectors φ_{ik} may be regarded as to vary at random across an individual mean parameter vector φ_i, which describes the general behaviour of the respective processes for that individual. Furthermore the individual mean processes are supposed to vary across a population mean process with parameter vector φ in the manner of a random sample. Additionally we suppose that the variances of the observed concentration-time curves differ from individual to individual and from occasion to occasion.

3.2 Model I

Nonlinear hierarchical model

A Bayesian approach according to Racine-Poon (1985) and Racine-Poon and Smith (1990) is applied to the data. We are interested especially in the variation of the individual responses at different dosing occasions, the so called *interoccasion* variability, and the variation between the individuals, the *intersubject* variability.

We propose a four-stage nonlinear hierarchical model assuming that our observations y_{ijk} of the concentration of ethylene in the atmosphere of the exposition system are independent and have the following distribution:

given β_{ik}, τ_{ik}^2: $\quad y_{ijk} \sim N(f(\beta_{ik}, t_j), \tau_{ik}^2) \quad i = 1, \ldots, I, j = 1, \ldots, J$ and $k = 1, \ldots, K,$

with $\quad \beta_{ik} = (\varphi_{ik}^T, y_{ik}(0))^T$ and $\varphi_{ik} = (k_{12ik}, k_{21ik}, k_{elik})^T$

given β_i, Ω_i: $\quad \beta_{ik} \sim N(\beta_i, \Omega_i) \qquad i = 1, \ldots, I$ and $k = 1, \ldots, K,$

with $\quad \beta_i = (\varphi_i^T, y_i(0))^T$ and $\varphi_i = (k_{12i}, k_{21i}, k_{eli})^T,$

given β, Σ: $\quad \beta_i \sim N(\beta, \Sigma) \qquad i = 1, \ldots, I,$

with $\quad \beta = (\varphi^T, y(0))^T$ and $\varphi = (k_{12}, k_{21}, k_{el})^T$

$p(\beta) \propto 1 \qquad \forall\, \beta \in \mathbb{R}^4.$

Linear hierarchical model

We obtain the Bayes estimates for the population mean and individual parameter vectors β, β_i, and β_{ik} by transforming the nonlinear hierarchical model into a linear one, such as provided by Lindley and Smith (1972). For that purpose the observations y_{ijk} are replaced by an "almost" sufficient statistic ζ_{ik} with

$$\zeta_{ik} \sim N(\beta_{ik}, \tau_{ik}^2 C_{ik}), \quad i = 1, \ldots, I, k = 1, \ldots, K.$$

For example, ζ_{ik} can be chosen as the mean of the posterior density of β_{ik} . In the case of uninformative priors for the variances τ_{ik}^2, the posterior distribution of β_{ik} can be well approximated by its likelihood, so that the maximum likelihood estimate of β_{ik} can be used as a good approximation for ζ_{ik} (Racine-Poon, 1985).

The resulting linear hierarchical model is given by:

given θ, V: $\zeta \sim N(\theta, V)$,

where $\zeta = (\zeta_{1,1}, \ldots, \zeta_{IK})^T$, $\theta = (\theta_1, \ldots, \theta_I)^T = (\beta_{1,1}, \ldots, \beta_{IK})^T$, $\theta_i = (\beta_{i1}, \ldots, \beta_{iK})^T$

and $V = diag\{\tau_{1,1}^2 C_{1,1}, \ldots, \tau_{IK}^2 C_{IK}\}$

given ψ, Ω: $\theta \sim N(Z_2\psi, \Omega)$,

where $\theta = (\beta_{1,1}, \ldots, \beta_{IK})^T$, $\psi = (\beta_1, \ldots, \beta_I)^T$,

$$\Omega = \begin{pmatrix} \Omega_1 & & & & \\ & \ddots & & 0 & \\ & & \Omega_1 & & \\ & & & \Omega_2 & \\ & 0 & & & \ddots & \\ & & & & & \Omega_I \end{pmatrix}, \text{ and } Z_2 = \begin{pmatrix} I & 0 & \cdots & \cdots & 0 \\ \vdots & \vdots & & & \vdots \\ I & 0 & & & \\ 0 & I & 0 & & \vdots \\ \vdots & \vdots & \vdots & & \\ 0 & I & 0 & & \vdots \\ \vdots & 0 & \ddots & & \\ & & & \ddots & 0 \\ \vdots & \vdots & & 0 & I \\ & & & \vdots & \vdots \\ 0 & 0 & \cdots & 0 & I \end{pmatrix} \text{ is a suitable design matrix.}$$

given β, Λ: $\psi \sim N(Z_3\beta, \Lambda)$, where $\psi = (\beta_1, \ldots, \beta_I)^T$, $\Lambda = diag\{\Sigma, \ldots, \Sigma\}$, and

$Z_3 = (I_4, \ldots, I_4)^T$ is a suitable design matrix,

$p(\beta) \propto 1, \qquad \forall \beta \in \mathbb{R}^4.$

The matrix $\left(\tau_{ik}^2 C_{ik}\right)^{-1}$ denotes the Information matrix (cf. Selinski and Urfer, 1998, for further details):

$$\left(\tau_{ik}^2 C_{ik}\right)^{-1} = E\left[-\frac{\partial^2}{\partial\beta_{ik}\partial\beta_{ik}^T} \ln L\left(y_{1,1,1}, \ldots, y_{IJK} \middle| \beta_{1,1}, \ldots, \beta_{IK}, \tau_{1,1}^2, \ldots, \tau_{IK}^2\right)\right] \tag{5}$$

First of all, we suppose that our concentration-time curves can be well approximated by first order kinetic processes, adapting the main idea of the approach of Becka (1998).

With the notation of chapter 2 the concentration-time curve in the exposition system is given by

$$f\left(\beta_{ik}, t_j\right) = y_{ik}(0) \cdot \left\{ \frac{\left(k_{12ik} v_{2ik}^{2/3} + \lambda_{1ik}\right) \exp\left\{\lambda_{2ik} t_j\right\} - \left(k_{21ik} v_{2ik}^{1/3} + \lambda_{2ik}\right) \exp\left\{\lambda_{1ik} t_j\right\}}{\left(\lambda_{1ik} - \lambda_{2ik}\right)} \right\}, \qquad (6)$$

where $v_{2ik} = \left(\frac{V_{2ik}}{1000}\right)$ depends on the volume of the *i*th rat at the *k*th occasion V_{2ik} and

$$\lambda_{1ik,2ik} = \frac{1}{2}\left\{ -\left(k_{12ik} v_{2ik}^{2/3} + k_{21ik} v_{2ik}^{1/3} + k_{elik} v_{2ik}\right) \pm \sqrt{\left(k_{12ik} v_{2ik}^{2/3} + k_{21ik} v_{2ik}^{1/3} + k_{elik} v_{2ik}\right)^2 - 4 k_{12ik} k_{elik} v_{2ik}^{5/3}} \right\}$$

with $\lambda_{2ik} < \lambda_{1ik} < 0$ (cf. Selinski and Urfer, 1998, for further details).

The vectors of parameters β_{ik} in (6) are substituted by their maximum likelihood estimates ζ_{ik}, $i = 1, \ldots, I$, $k = 1, \ldots, K$.

Estimators in the case of unknown covariance matrices

In case of known variances τ_{ik}^2 and covariance matrices Ω_i , , $i = 1, \ldots, I$, $k = 1, \ldots, K$, and Σ Bayes estimates can be calculated following the approach of Lindley and Smith (1972) (Selinski, 2000). However, we have only vague knowledge about these covariance matrices, and the aim of our investigation is to gain information about just these covariances, especially with regard to the interoccasion and interindividual variability. Hence, we estimate both the parameter vectors and the covariance matrices using an EM algorithm as proposed by Dempster et al. (1977).

Racine-Poon and Smith (1990) suggest to replace unknown variances τ_{ik}^2, $i = 1, \ldots, I$, $k = 1, \ldots, K$, by suitable estimates $\hat{\tau}_{ik}^2$. Under the assumptions of our model and furthermore assuming independent variances τ_{ik}^2 with vague prior distribution $p(\tau_{ik}^2) \propto 1$, the posterior mode of τ_{ik}^2 is equivalent to its maximum likelihood estimate $\hat{\tau}_{ik}^2$. Thus, we approximate the Bayes estimate of τ_{ik}^2 by

$$\hat{\tau}_{ik}^2 = \frac{1}{J} \cdot \sum_{j=1}^{J} \left(y_{ijk} - f\left(\zeta_{ik}, t_j\right)\right)^2 \quad , i = 1, \ldots, I, k = 1, \ldots, K. \tag{7}$$

Assuming that the inverse covariance matrices Ω_i^{-1}, $i = 1, \ldots, I$, and Σ^{-1} follow Wishart distributions with degrees of freedom ρ_1 and ρ_2 and matrices R_1 and R_2, respectively, $R_1^{-1}/(\rho_1 - p-1)$ and $R_2^{-1}/(\rho_2 - p-1)$ play the role of prior estimates of Ω_i and Σ and the joint posterior density for $\beta_{1,1}, \ldots, \beta_{IK}, \beta_1, \ldots, \beta_I, \beta, \Omega_1^{-1}, \ldots, \Omega_I^{-1}$ and Σ^{-1}, given $\zeta_{1,1}, \ldots, \zeta_{IK}$, is proportional to

$$\begin{aligned}
&\left(\prod_{i=1}^{I}\prod_{k=1}^{K}\left|\hat{\tau}_{ik}^2 C_{ik}\right|^{-1/2}\right) \cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}\sum_{k=1}^{K}\frac{1}{\hat{\tau}_{ik}^2}(\zeta_{ik} - \beta_{ik})^T \cdot C_{ik}^{-1}(\zeta_{ik} - \beta_{ik})\right\} \cdot \\
&\left(\prod_{i=1}^{I}|\Omega_i|^{-K/2}\right) \cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}\sum_{k=1}^{K}(\beta_{ik} - \beta_i)^T \Omega_i^{-1}(\beta_{ik} - \beta_i)\right\} \cdot \\
&|\Sigma|^{-I/2} \cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}(\beta_i - \beta)^T \Sigma^{-1}(\beta_i - \beta)\right\} \cdot \\
&\prod_{i=1}^{I}|\Omega_i|^{-1/2(\rho_1 - p - 1)} \cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I} tr\left(R_1^{-1} \cdot \Omega_i^{-1}\right)\right\} \cdot \\
&|\Sigma|^{-1/2(\rho_2 - p - 1)} \cdot \exp\left\{-\frac{1}{2} tr\left(R_2^{-1} \cdot \Sigma^{-1}\right)\right\}
\end{aligned} \tag{8}$$

Vague knowledge about the inverse covariance matrices $\Omega_1^{-1}, \ldots, \Omega_I^{-1}$, and Σ^{-1} can be expressed by choosing ρ_1 and ρ_2 as small as possible, i. e. $\rho_1 = \rho_2 = p = 4$. The choice of R_1 and R_2, respectively, seems to have little influence on the estimates (Racine-Poon, 1985).

Substituting $\hat{\tau}_{ik}^2$ for τ_{ik}^2, if necessary, we obtain the approximations of the Bayes estimates at the lth iteration of the EM algorithm, $\beta^{(l)}$, $\psi^{(l)} = \left(\beta_1^{(l)}, \ldots, \beta_I^{(l)}\right)^T$, and

$\theta^{(l)} = \left(\beta_{1,1}^{(l)}, \ldots, \beta_{IK}^{(l)}\right)^T$, by replacing the covariance matrices by their current approximations $\Omega^{(l-1)}$, and $\Lambda^{(l-1)}$ (E-Step) and subsequent calculation of $\Omega^{(l)}$ and $\Lambda^{(l)}$ as the posterior mode of (8) using $\beta^{(l)}$, $\psi^{(l)}$, and $\theta^{(l)}$ (M-Step) (Selinski, 2000).

<u>E-Step</u>

Approximating Ω and Λ by $\Omega^{(l-1)}$ and $\Lambda^{(l-1)}$ we obtain

$$\beta^{(l)} = \left[Z_3^T Z_2^T \left\{ \hat{V} + \Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} Z_2 Z_3 \right]^{-1} Z_3^T Z_2^T \left\{ \hat{V} + \Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} \zeta \,, \tag{9}$$

where $\hat{V} = diag\left\{ \left(\hat{\tau}_{ik}^2 C_{ik} \right), \ldots, \left(\hat{\tau}_{ik}^2 C_{ik} \right) \right\}$.

Substituting β, Ω, and Λ by $\beta^{(l)}$, $\Omega^{(l-1)}$, and $\Lambda^{(l-1)}$, respectively, yields

$$\psi^{(l)} = \left[Z_2^T \left(\hat{V} + \Omega^{(l-1)} \right)^{-1} Z_2 + \Lambda^{(l-1)^{-1}} \right]^{-1} \left[Z_2^T \left(\hat{V} + \Omega^{(l-1)} \right)^{-1} \zeta + \Lambda^{(l-1)^{-1}} Z_3 \beta^{(l-1)} \right]. \tag{10}$$

In the same way we get $\theta^{(l)}$ by replacing the unknown parameters by their current estimates:

$$\theta^{(l)} = \left[\hat{V}^{-1} + \left\{ \Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} \right]^{-1} \left[\hat{V}^{-1} \zeta + \left\{ \Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} Z_2 Z_3 \beta^{(l)} \right]. \tag{11}$$

M-Step

Setting β, ψ and θ equal to their current values $\beta^{(l)}$, $\psi^{(l)} = \left(\beta_1^{(l)},\ldots,\beta_I^{(l)}\right)^T$ and $\theta^{(l)} = \left(\beta_{1,1}^{(l)},\ldots,\beta_{IK}^{(l)}\right)^T$ the conditional posterior mode of (8) is given by

$$\Omega_i^{(l)} = \frac{R_1^{-1} + \sum_{k=1}^{K}\left(\beta_{ik}^{(l)} - \beta_i^{(l)}\right)\cdot\left(\beta_{ik}^{(l)} - \beta_i^{(l)}\right)^T}{K + \rho_1 - p - 1}, \qquad i = 1, \ldots, I, \text{ and} \tag{12}$$

$$\Sigma^{(l)} = \frac{R_2^{-1} + \sum_{i=1}^{I}\left(\beta_i^{(l)} - \beta^{(l)}\right)\left(\beta_i^{(l)} - \beta^{(l)}\right)^T}{I + \rho_2 - p - 1} \tag{13}$$

Both steps are repeated until $\Omega_1^{(l)}$, . . ., $\Omega_I^{(l)}$, and $\Sigma^{(l)}$ converge. Racine-Poon (1985) suggests as criterion for convergence, that the maximum change in the elements of the covariance matrices between successive iterations should be less than 0.001.

Reasonable starting values $\Omega_1^{(0)}$, . . ., $\Omega_I^{(0)}$, and $\Sigma^{(0)}$ are given by

$$\Omega_i^{(0)} = \frac{R_1^{-1} + \sum_{k=1}^{K}\left(\zeta_{ik} - \overline{\zeta}_{i.}\right)\left(\zeta_{ik} - \overline{\zeta}_{i.}\right)^T}{K + \rho_2 - p - 2}, \qquad i = 1, \ldots, I$$

$$\Sigma^{(0)} = \frac{R_2^{-1} + \sum_{i=1}^{I}\left(\overline{\zeta}_{i.} - \overline{\zeta}_{..}\right)\left(\overline{\zeta}_{i.} - \overline{\zeta}_{..}\right)^T}{I + \rho_2 - p - 3},$$

where $\overline{\zeta}_{i.} = \frac{1}{K}\sum_{k=1}^{K}\zeta_{ik}$ and $\overline{\zeta}_{..} = \frac{1}{I}\sum_{i=1}^{I}\overline{\zeta}_{i.} = \frac{1}{IK}\sum_{i=1}^{I}\sum_{k=1}^{K}\zeta_{ik}$.

3.3 Model II

The estimation of the parameter vectors and covariance matrices of Model I requires the manipulation of quadratic matrices of size $p \cdot I \cdot K$. This leads to computational problems due to the size and conditioning of the matrices. In a design using only ten animals, five replications, and four parameters, matrices of size (200 x 200) have to be repeatedly manipulated at each iteration of the EM-algorithm. These operations, including multiplication and inversion of the matrices lead to numerically questionable results due to the large number of operations and the representational errors in the computer memory, i.e. the mantissa being cut off after a certain number of digits. The latter results in a difference between a number and its digital representation in the computer (e.g. 1/3 can never be accurately represented by 0.33333...). The effect is magnified by the repeated computing operations. Hence, we propose a less complex model, Model II, which ignores the correlation between the individual and occasion-dependent parameter vectors β_{ik} .This model allows an estimation of the parameters by manipulating matrices of size p x p, which is independent of the number of animals and measurements in the experiment.

The nonlinear four-stage hierarchical model is the same as given in the previous section. Additionally the parameter vectors β_{i1}, . . ., β_{iK} are assumed to be independent. The transformation to the linear model is performed by substituting the observation y_{ijk} in the first stage by the Maximum-Likelihood estimates of the parameter vectors β_{ik}.. Thus, we obtain the following linear hierarchical model.

Linear hierarchical model

given β_{ik}, τ_{ik}^2: $\zeta_{ik} \sim N(\beta_{ik}, \tau_{ik}^2 C_{ik})$, $i = 1, \ldots, I, k = 1, \ldots, K$

given β_i, Ω_i: $\beta_{ik} \sim N(\beta_i, \Omega_i)$, $i = 1, \ldots, I, k = 1, \ldots, K$

given β, Σ: $\beta_i \sim N(\beta, \Sigma)$, $i = 1, \ldots, I$

$p(\beta) \propto 1$, $\forall \beta \in \mathbb{R}^4$.

where $\tau_{ik}^{-2} C_{ik}^{-1}$ is the Fisher Information matrix:

$$\tau_{ik}^{-2} C_{ik}^{-1} = E\left[-\frac{\partial^2}{\partial\beta_{ik}\partial\beta_{ik}^T} \ln L\left(y_{1,1,1}, \ldots, y_{10,J,5} \middle| \beta_{1,1}, \ldots, \beta_{10,5}, \tau_{1,1}^2, \ldots, \tau_{10,5}^2\right)\right]$$

EM algorithm

With the assumptions of section 3.2 for τ_{ik}^2, Ω_i^{-1}, and Σ^{-1}, furthermore, assuming independence between $\beta_{i1}, \ldots, \beta_{iK}$ the EM algorithm is performed as follows:

E-Step

Approximating $\Omega_1, \ldots, \Omega_I, \Sigma$ by $\Omega_1^{(l-1)}, \ldots, \Omega_I^{(l-1)}$, and $\Sigma^{(l-1)}$ we obtain

$$\beta^{(l)} = \left[\sum_{i=1}^{I}\sum_{k=1}^{K}\left(\hat{\tau}_{ik}^2 C_{ik} + \Omega_i^{(l-1)} + \Sigma^{(l-1)}\right)^{-1}\right]^{-1} \cdot \sum_{i=1}^{I}\sum_{k=1}^{K}\left(\hat{\tau}_{ik}^2 C_{ik} + \Omega_i^{(l-1)} + \Sigma^{(l-1)}\right)^{-1} \zeta_{ik} \qquad (14)$$

Furthermore, substituting β by $\beta^{(l)}$ yields

$$\beta_i^{(l)} = \left[\left[\sum_{k=1}^{K}\left(\hat{\tau}_{ik}^2 C_{ik} + \Omega_i^{(l-1)}\right)^{-1}\right] + \Sigma^{(l-1)^{-1}}\right]^{-1} \cdot \left[\left(\sum_{k=1}^{K}\left(\hat{\tau}_{ik}^2 C_{ik} + \Omega_i^{(l-1)}\right)^{-1} \cdot \zeta_{ik}\right) + \Sigma^{(l-1)^{-1}} \cdot \beta^{(l)}\right] \qquad (15)$$

In the same way we get $\beta_{ik}^{(l)}$ by replacing the unknown parameters by their current estimates:

$$\beta_{ik}^{(l)} = \left[\left(\hat{\tau}_{ik}^{2} C_{ik} \right)^{-1} + \left(\Omega_i^{(l-1)} + \Sigma^{(l-1)} \right)^{-1} \right]^{-1} \cdot \left[\left(\hat{\tau}_{ik}^{2} C_{ik} \right)^{-1} \cdot \zeta_{ik} + \left(\Omega_i^{(l-1)} + \Sigma^{(l-1)} \right)^{-1} \cdot \beta^{(l)} \right]. \tag{16}$$

M-Step

Conditioning on $\beta_{ik} = \beta_{ik}^{(l)}$, $\beta_i = \beta_i^{(l)}$ and $\beta = \beta^{(l)}$, i = 1, . . ., I, k = 1, . . ., K, the conditional posterior mode is given by

$$\Omega_i^{(l)} = \frac{R_1^{-1} + \sum_{k=1}^{K} \left(\beta_{ik}^{(l)} - \beta_i^{(l)} \right) \cdot \left(\beta_{ik}^{(l)} - \beta_i^{(l)} \right)^T}{K + \rho_1 - p - 1}, \quad i = 1, \ldots, I, \text{ and} \tag{17}$$

$$\Sigma^{(l)} = \frac{R_2^{-1} + \sum_{i=1}^{I} \left(\beta_i^{(l)} - \beta^{(l)} \right) \left(\beta_i^{(l)} - \beta^{(l)} \right)^T}{I + \rho_2 - p - 1} \tag{18}$$

Both steps are repeated until $\Omega_1^{(l)}, \ldots, \Omega_I^{(l)}$, and $\Sigma^{(l)}$ converge, i. e., that the maximum change in the elements of the covariance matrices between successive iterations should be less than 0.001.

Reasonable starting values $\Omega_1^{(0)}, \ldots, \Omega_I^{(0)}$, and $\Sigma^{(0)}$ are given by

$$\Omega_i^{(0)} = \frac{R_1^{-1} + \sum_{k=1}^{K} \left(\zeta_{ik} - \overline{\zeta}_{i.} \right) \left(\zeta_{ik} - \overline{\zeta}_{i.} \right)^T}{K + \rho_2 - p - 2}, \quad i = 1, \ldots, I, \text{ and}$$

$$\Sigma^{(0)} = \frac{R_2^{-1} + \sum_{i=1}^{I} \left(\overline{\zeta}_{i.} - \overline{\zeta}_{..} \right) \left(\overline{\zeta}_{i.} - \overline{\zeta}_{..} \right)^T}{I + \rho_2 - p - 3},$$

where $\overline{\zeta}_{i.} = \frac{1}{5} \sum_{k=1}^{K} \zeta_{ik}$ and $\overline{\zeta}_{..} = \frac{1}{I} \sum_{i=1}^{I} \overline{\zeta}_{i.} = \frac{1}{IK} \sum_{i=1}^{I} \sum_{k=1}^{K} \zeta_{ik}$.

5. Results

The methods presented in the previous chapters were applied to data from an inhalation experiment mentioned in section 1. Both, Model I and Model II were implemented for the first goup using SAS® (Schirm, 1999). In case of normality, the maximum likelihood estimates ζ_{ik} coincide with the least squares estimates. Thus, ζ_{ik} can be conveniently estimated using PROC NLIN. This procedure provides also an estimation of the variances τ_{ik}^2, being the mean squared residuals of the least squares estimation (see eq. (7)). The least squares estimators transport the information from the experiment into the hierarchical model, thus yielding a first look at the estimates. Table A.1 (appendix) shows the least squares estimates provided by the Marquardt-algorithm in the PROC NLIN procedure (SAS STAT users guide, 1994). The means squared residuals in Table A.2 provide both, the estimate of the variance $\hat{\tau}_{ik}^2$, and a measurement for the fit of the linear model, allowing the detection of possible outliers (e.g. animal 3, day 2).

Animal 10 was dropped out of the experiment during day 4 because of health problems not related to the experiment.

5.1. Model I

As mentioned before, the computation of estimates for Model I is connected with possibly large numerical errors. In tables B.1 – B.3 (appendix) the Bayes estimates for the population parameters computed by implementing equations (9) – (13) of the EM-algorithm in SAS/IML® are shown. While the estimate for the population mean vector in table B.1 seems to be consistent with the raw estimates in table A.1, the estimates for individual mean parameters and the specific parameters are questionable. All the individual estimates are almost identical to their respective population means, and the estimates for the velocity constant for the uptake, $\hat{k}_{12}$, are negative in 9 out of 10 animals. While possible in the mathematical model, a negative uptake, i.e. exhalation of endogeneously produced ethylene and elimination of ethylene by other means at this rate is not sensible for toxicological reasons. The estimates for the individual covariances Ω_i indicate an almost uniform variance for all components of the parameter vector, which points to numerical problems in the computations of the estimate. The estimate for the population covariance Σ is obviously distorted.

5.2. Model II

Alternatively, equations (14) – (18) were implemented, also using SAS/IML®. The algorithm needed only about a third of the iterations compared to the EM algorithm of Model I, giving this model a numerical advantage because the number of computing operations is greatly reduced. Table 1 – 3 show the estimators of the population mean, individual mean and specific kinetic constants β, β_i, and β_{ik}, $i = 1, \ldots, 10$, $k = 1, \ldots, 5$, based on Model II.

Table 1: *Estimated population mean parameters from Model II.*

$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
0,0195	1,9459	7,9203	120,7751

Table 2: *Estimated individual mean parameters from Model II.*

rat	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
1	0,0165	1,7996	8,2271	122,0978
2	0,0251	1,4295	9,7140	125,9349
3	0,0172	1,6769	8,6843	123,6201
4	0,0170	1,5062	9,4373	124,6642
5	0,0395	2,7110	5,9216	109,8376
6	0,0152	1,8898	7,8890	121,3839
7	0,0153	1,7540	8,3365	122,1477
8	0,0185	2,3801	6,5166	116,9047
9	0,0171	2,1202	7,2037	119,2689
10	0,0163	1,9532	7,6405	120,7579

Table 3: *Estimated individual occasion-dependent parameters from Model II.*

rat	day	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$	rat	day	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
1	1	0,0179	2,0424	7,8139	120,4073	6	1	0,0158	1,4976	8,4349	122,7631
1	2	0,0370	1,1774	8,3388	121,9544	6	2	0,0148	0,5846	9,3530	126,5864
1	3	0,0276	3,7447	5,9832	115,5819	6	3	0,0146	1,5965	8,4010	122,6981
1	4	0,0173	2,8074	7,2129	118,9648	6	4	0,0152	1,9571	7,9596	120,9847
1	5	0,0161	1,7541	8,2190	121,9360	6	5	0,0145	1,9141	8,0283	121,2633
2	1	0,0162	1,8132	8,3107	122,2961	7	1	0,0177	1,2889	8,4888	122,8876
2	2	0,0157	0,5766	10,1476	129,1667	7	2	0,0178	1,8176	8,0158	121,0803
2	3	0,0288	2,9114	6,9585	118,2023	7	3	0,0152	1,8046	8,0610	121,2859
2	4	0,0241	0,9839	8,9140	124,2524	7	4	0,0127	2,4287	7,3941	118,9533
2	5	0,0272	1,1130	8,5302	122,7155	7	5	0,0147	1,6953	8,2006	121,8441
3	1	0,0139	1,2868	9,0321	125,3350	8	1	0,0190	1,2020	8,9751	124,4785
3	2	0,0386	1,4377	8,1966	121,5839	8	2	0,0189	1,3019	8,9513	124,4497
3	3	0,0200	2,6895	6,8662	116,9364	8	3	0,0197	1,4846	8,7033	123,5732
3	4	0,0201	2,3987	7,3475	118,7158	8	4	0,0189	2,6431	6,6844	116,5329
3	5	0,0171	1,5405	8,2975	122,1170	8	5	0,0174	2,7736	6,8947	117,7432
4	1	0,0168	2,3980	7,1087	118,1416	9	1	0,0186	2,8072	6,8077	116,7688
4	2	0,0161	2,0931	7,7696	120,3665	9	2	0,0218	3,3662	6,7982	118,2781
4	3	0,0189	2,8631	6,1714	115,1470	9	3	0,0181	2,2097	7,7350	120,2433
4	4	0,0168	1,9826	7,9291	120,8590	9	4	0,0163	1,6759	8,2920	122,2700
4	5	0,0171	1,2532	9,1140	124,8166	9	5	0,0164	2,2476	7,5441	119,3607
5	1	0,0151	1,3722	8,6944	124,4412	10	1	0,0210	1,4504	8,3251	122,2795
5	2	0,0170	2,5219	7,1380	117,3994	10	2	0,0261	2,6438	6,9201	116,7731
5	3	0,0170	1,6030	8,3336	122,6333	10	3	0,0201	3,1723	6,3442	115,0667
5	4	0,0290	3,9191	5,5637	113,4746	10	4	0,0160	2,0168	7,8933	120,7383
5	5	0,0495	3,5087	6,2935	115,8091	10	5	---	---	---	---

These results appear to be more stable from a numeric point of view and are also far more consistent with the maximum-likelihood estimates in table A.1 In general, extreme data points in some components are corrected towards a common mean by the Bayes estimation; e.g. animal 5, day 4 and 5 with low maximum-likelihood estimates of initial concentration and velocity constant for elimination, $\hat{k}_{el}$, and high estimates of maximum-likelihood estimates for uptake, $\hat{k}_{12}$, are corrected towards the mean.

As mentioned above, a comparison of the interindividual and interoccasional variability can be made by computing estimates of the covariance matrices Ω_i, i = 1, . . . , 10 and Σ .

Table 4: *Estimates of the individual covariance matrices* Ω_i *in Model II.*

rat				
1	1,1112	0,0011	-0,0027	-0,0088
	0,0011	1,6942	-0,6174	-1,7940
	-0,0027	-0,6174	1,8052	2,0536
	-0,0088	-1,7940	2,0536	7,2421
2	1,1111	0,0011	-0,0004	-0,0035
	0,0011	1,4855	-0,4734	-1,5382
	-0,0004	-0,4734	2,4212	3,6635
	-0,0035	-1,5382	3,6635	11,8527
3	1,1112	0,0001	-0,0023	-0,0092
	0,0001	1,3083	-0,3080	-1,1427
	-0,0023	-0,3080	1,7335	2,3199
	-0,0092	-1,1427	2,3199	9,7854
4	1,1111	0,0002	-0,0005	-0,0014
	0,0002	1,4746	-0,9026	-2,5671
	-0,0005	-0,9026	3,4722	6,5698
	-0,0014	-2,5671	6,5698	19,5659
5	1,1113	0,0064	-0,0157	-0,0880
	0,0064	1,6835	-0,7500	-2,8888
	-0,0157	-0,7500	2,8058	9,0527
	-0,0880	-2,8888	9,0527	54,7850
6	1,1111	0,0000	0,0000	-0,0002
	0,0000	1,3276	-0,2519	-0,8607
	0,0000	-0,2519	1,4142	0,9997
	-0,0002	-0,8607	0,9997	4,5410
7	1,1111	-0,0003	0,0002	0,0008
	-0,0003	1,1869	-0,0815	-0,2881
	0,0002	-0,0815	1,2343	0,4160
	0,0008	-0,2881	0,4160	2,5251
8	1,1111	-0,0003	0,0005	0,0014
	-0,0003	1,5085	-0,8097	-2,5331
	0,0005	-0,8097	2,9916	5,7585
	0,0014	-2,5331	5,7585	18,8444
9	1,1111	0,0008	-0,0003	-0,0011
	0,0008	1,3607	-0,1300	-0,4652
	-0,0003	-0,1300	1,3226	0,5785
	-0,0011	-0,4652	0,5785	3,0218
10	1,1111	0,0010	-0,0010	-0,0059
	0,0010	1,3578	-0,2673	-1,1618
	-0,0010	-0,2673	1,4147	1,2539
	-0,0059	-1,1618	1,2539	6,7316

Table 5: *Estimates of the population covariance matrix Σ in Model II.*

$\hat{\Sigma}$				
	1,1112	0,0016	-0,0038	-0,0235
	0,0016	1,2665	-0,4653	-1,7714
	-0,0038	-0,4653	2,5475	5,1850
	-0,0235	-1,7714	5,1850	22,3028

The results indicate a correlation between the rate of elimination and initial concentration of ethene, which is not consistent with the assumption of a first order kinetic.

The next step after the computation of the estimators is to answer the question of their "quality" according to different criteria. A general discussion of the analytical properties of Bayes estimates in a hierarchical normal model is omitted in this paper.

However, if we want to apply the estimators of the population parameters, it might be useful to compute the variation coefficient, i.e. to express the standard deviation of an estimate in multiples of its mean. By computing these figures, it is also possible to answer the question if interoccasional or interindividual variability is higher.

Table 6: *Estimated variation coefficients of the population mean vector β (Model II).*

v($\hat{k}_{12}$)	v($\hat{k}_{21}$)	v($\hat{k}_{el}$)	v($\hat{y}(0)$)
54,1379	0,5789	0,2017	0,0347

Table 7: *Estimated variation coefficients of the individual mean vectors* β_i *(Model II)*

rat	v($\hat{k}_{12}$)	v($\hat{k}_{21}$)	v($\hat{k}_{el}$)	v($\hat{y}(0)$)
1	63,6431	0,7240	0,1635	0,0198
2	41,8743	0,8560	1,0161	0,0254
3	61,2824	0,6812	0,1532	0,0236
4	62,2581	0,8039	0,1996	0,0342
5	26,5692	0,4763	0,2878	0,0580
6	69,3984	0,6108	0,1520	0,0170
7	68,9671	0,6220	0,1335	0,0124
8	56,7999	0,5153	0,2674	0,0351
9	61,5637	0,5491	0,1600	0,0139
10	64,6053	0,5952	0,1570	0,0202

From tables 6 and 7, the kinetic constants can be divided into two groups. The first group consists of the estimates for the constants for uptake and exhalation, $\hat{k}_{12}$ and $\hat{k}_{21}$. The interoccasion variability in general is greater than the interindividual variability for these two constants. The other group consists of the estimates for the elimination rate, $\hat{k}_{el}$, where the interindividual variability is greater than the interoccasion variability.

A possible explanation for this behaviour of the rates is the fact, that $\hat{k}_{12}$ and $\hat{k}_{21}$ are estimates of the constants of the interface between the organism an the environment, while $\hat{k}_{el}$ is an estimate for the endogenous elimination of the xenobiotic and therefore being influenced less by environmental factors.

6. Discussion

The present approach simplifies the complex biological processes of highly organised living organisms by the reduction to two-compartment models and the approximation of nonlinear kinetics by linear ones. Using linear kinetics we have to be aware of the possible errors which result from the dependence of the parameters on the concentration if the underlying processes are nonlinear. Assuming first order kinetics the processes of uptake, exhalation, and metabolic elimination are independent from the dose. Before summarising the information provided by experiments within a range of concentrations, like in the experiments of group B, it is necessary to verify that a first order approximation of the processes is valid. In fact, the experiments of group A show a correlation between the metabolism and the initial concentration. In a further paper a procedure will be presented to detect such critical departures from linearity.

Implementing the model in a computer using SAS/IML®, we experienced severe numerical difficulties, especially with Model I, when trying to invert large almost singular matrices, which were present in the computation of the population parameter estimates. Even though the EM-type algorithm converged after some modification of the program algorithm, the results of model 1 are numerically questionable at best.

Model II, while neglecting some aspects of the covariance structure of the parameter vectors, has the advantage to be computable by a numerically stable algorithm and therefore yielding numerically more accurate results.

Determining the processes involved in the formation of reactive metabolites is a crucial step to establish a dose-response relationship for the interesting chemical. The metabolites may be transformed partly into an inactive form, and others form various DNA, RNA, and protein adducts. These processes may also contribute to the nonlinearity of the dose-tumor response curve. Hoel et al. (1983) presume a linear DNA adduct–tumor relation and conclude that a valid characterisation of the processes of uptake, elimination, and metabolism is a necessary part of the risk assessment of potential mutagens and carcinogens.

Various attempts have been published to determine toxicokinetic parameters. Holländer et al. (1998) compared log-linear regression, a *noncompartmental* method, unweighted and weighted nonlinear least squares regression, *multicompartmental* methods, using different weighting schemes. They found that the parameters depended on the model and the weighting scheme and stressed the importance of correct assumptions with respect to the variability, presenting an approach to use information about the analytical method in order to estimate the variability of the observation.

Gilberg et al. (1999) discussed an extension of the nonlinear random effects model for the Michaelis-Menten enzyme kinetic by adding a flexible transformation to both sides of the model. The so called weighted transform-both-sides models are very adaptable with respect to the error structure. An EM algorithm, which updates the transformation and weighting parameters every iteration step, was applied to estimate regression and covariance parameters.

Toxicological data reflect profound complexities of the biology of living individuals. Recent research on Gibbs sampling has great potential for estimating the parameters of complex models, because it reduces the problem of dealing simultaneously with a large number of related parameters into a much simpler problem of dealing with one unknown quantity at a time. Gilks et al. (1993) have reviewed applications of Gibbs sampling in immunology, pharmacology, cancer screening, industrial and genetic epidemiology. Wikle et al. (1998) propose the use of hierarchical Bayesian space-time model with five stages to achieve more flexible models and methods for the analysis of environmental data distributed in space and time. They implement their models in a Markov Chain Monte Carlo framework using the Gibbs sampler approach. Increasing familiarity and experimentation with new Markov Chain Monte Carlo methods for exploring and summarising posterior distributions in Bayesian statistics will lead to new insights in toxicokinetics.

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Appendix

A. Maximum Likelihood Estimates

Table A.1: *Maximum-Likelihood/Least Squares estimators ζ_{ik} for the kinetic parameters*

rat	day	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
1	1	0,0179	1,9834	7,5627	120,3721
	2	0,0370	1,3815	10,1214	122,4654
	3	0,0282	3,0936	4,7398	111,6025
	4	0,0175	2,4801	6,2660	115,8488
	5	0,0161	1,7670	8,2543	122,1925
2	1	0,0162	1,7919	8,1693	122,5419
	2	0,0156	0,8078	13,4555	130,3897
	3	0,0290	2,5768	6,0722	114,4934
	4	0,0240	1,2132	10,6692	127,2511
	5	0,0271	1,3280	10,0242	126,5720
3	1	0,0138	1,3979	9,6647	126,1174
	2	0,0388	1,5373	9,3229	122,7035
	3	0,0201	2,4761	6,2576	116,1434
	4	0,0202	2,2535	6,8527	118,3251
	5	0,0170	1,6386	8,7623	123,8729
4	1	0,0169	2,2823	6,7253	117,9545
	2	0,0161	2,0188	7,4552	120,2150
	3	0,0190	2,6849	5,7432	114,2223
	4	0,0168	1,9370	7,7216	120,8827
	5	0,0171	1,3649	9,8498	125,5488
5	1	0,0151	1,4682	9,3428	124,5229
	2	0,0170	2,3446	6,5673	117,2172
	3	0,0170	1,6582	8,6420	122,7099
	4	0,0299	3,0153	3,9671	109,8855
	5	0,0501	2,9668	5,0905	104,1462
6	1	0,0157	1,5901	8,8894	123,5081
	2	0,0146	0,8859	12,7375	128,9003
	3	0,0146	1,6536	8,6472	123,2277
	4	0,0152	1,9170	7,7655	121,0803
	5	0,0145	1,8831	7,8644	121,4114
7	1	0,0176	1,4496	9,4554	124,0585
	2	0,0178	1,8279	8,0623	121,1315
	3	0,0152	1,8132	8,0818	121,4740
	4	0,0128	2,2423	6,8069	118,2284
	5	0,0147	1,7339	8,3582	122,1776
8	1	0,0190	1,3459	9,9516	125,5356
	2	0,0188	1,4119	9,6450	125,2320
	3	0,0196	1,5525	9,0676	124,1400

	4	0,0190	2,4840	6,2286	116,1028
	5	0,0176	2,5093	6,1872	116,1285
9	1	0,0187	2,5403	6,1001	115,4773
	2	0,0221	2,8076	5,4744	113,2601
	3	0,0182	2,0875	7,2773	119,6566
	4	0,0162	1,7161	8,4109	122,9679
	5	0,0164	2,1371	7,1308	119,2333
10	1	0,0210	1,5697	9,0092	122,9741
	2	0,0263	2,4504	6,3709	115,3474
	3	0,0203	2,7899	5,4678	113,5891
	4	0,0160	1,9540	7,6131	120,7674

Table A.2: *Mean squared residuals $\hat{\tau}_{ik}^2$ of the least squares model.*

rat	day	Number of Obsevations	$\hat{\tau}_{ik}^2$	rat	day	number of obsevations	$\hat{\tau}_{ik}^2$
1	1	19	0,3751	6	1	21	1,5588
	2	20	7,6993		2	20	1,8800
	3	21	2,3942		3	21	1,1651
	4	20	1,1378		4	21	1,1731
	5	20	0,8222		5	21	1,0177
2	1	19	0,5984	7	1	20	1,9394
	2	20	0,7304		2	21	0,8443
	3	21	8,0063		3	19	1,5757
	4	21	2,6939		4	21	2,1700
	5	21	8,8819		5	20	1,2412
3	1	19	0,8810	8	1	20	1,4161
	2	20	46,7935		2	19	0,9936
	3	21	0,7284		3	19	0,8557
	4	21	0,7362		4	21	0,4737
	5	19	1,5714		5	20	5,9009
4	1	19	0,3881	9	1	21	0,9357
	2	20	16,7085		2	19	6,3617
	3	21	0,7776		3	19	8,7399
	4	21	0,4518		4	20	2,1776
	5	21	1,0244		5	21	0,3247
5	1	19	0,2953	10	1	21	1,5953
	2	20	0,6920		2	21	0,5121
	3	21	0,5447		3	21	0,7565
	4	20	1,5790		4	16	0,6898
	5	18	0,9940		5	0	---

B. Bayes Estimates, Model I

Table B.1: *Estimated population mean parameters from Model I.*

$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
0,0207	2,2663	8,7248	121,3182

Table B.2: *Estimated individual mean parameters from Model I.*

rat	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
1	-0,1245	2,1213	8,5791	121,1731
2	0,2499	2,4954	8,9546	121,5473
3	-0,0660	2,1798	8,6379	121,2316
4	-0,0514	2,1943	8,6525	121,2462
5	-0,1194	2,1263	8,5842	121,1782
6	0,1691	2,4145	8,8736	121,4665
7	-0,0843	2,1613	8,6195	121,2132
8	-0,0338	2,2119	8,6702	121,2638
9	0,0704	2,3161	8,7746	121,3679
10	-0,0095	2,2363	8,6946	121,2882

Table B.3 *Estimated individual mean parameters for Model I.*

rat	day	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$	rat	day	$\hat{k}_{12}$	$\hat{k}_{21}$	$\hat{k}_{el}$	$\hat{y}(0)$
1	1	0,0207	2,2663	8,7248	121,3182	6	1	0,0207	2,2663	8,7248	121,3182
1	2	0,0207	2,2663	8,7248	121,3182	6	2	0,0207	2,2663	8,7248	121,3182
1	3	0,0207	2,2663	8,7248	121,3182	6	3	0,0207	2,2663	8,7248	121,3182
1	4	0,0207	2,2663	8,7248	121,3182	6	4	0,0207	2,2663	8,7248	121,3182
1	5	0,0207	2,2663	8,7248	121,3182	6	5	0,0207	2,2663	8,7248	121,3182
2	1	0,0207	2,2663	8,7248	121,3182	7	1	0,0207	2,2663	8,7248	121,3182
2	2	0,0207	2,2663	8,7248	121,3182	7	2	0,0207	2,2663	8,7248	121,3182
2	3	0,0207	2,2663	8,7248	121,3182	7	3	0,0207	2,2663	8,7248	121,3182
2	4	0,0207	2,2663	8,7248	121,3182	7	4	0,0207	2,2663	8,7248	121,3182
2	5	0,0207	2,2663	8,7248	121,3182	7	5	0,0207	2,2663	8,7248	121,3182
3	1	0,0208	2,2665	8,7249	121,3184	8	1	0,0207	2,2663	8,7248	121,3182
3	2	0,0208	2,2665	8,7249	121,3188	8	2	0,0207	2,2663	8,7248	121,3182
3	3	0,0208	2,2665	8,7249	121,3184	8	3	0,0207	2,2663	8,7248	121,3182
3	4	0,0208	2,2665	8,7249	121,3184	8	4	0,0207	2,2663	8,7248	121,3182
3	5	0,0208	2,2665	8,7249	121,3184	8	5	0,0207	2,2663	8,7248	121,3182
4	1	0,0207	2,2663	8,7248	121,3182	9	1	0,0174	2,2631	8,7215	121,3149
4	2	0,0207	2,2663	8,7248	121,3182	9	2	0,0174	2,2630	8,7215	121,3025
4	3	0,0207	2,2663	8,7248	121,3182	9	3	0,0174	2,2631	8,7215	121,3149
4	4	0,0207	2,2663	8,7248	121,3182	9	4	0,0174	2,2631	8,7215	121,3149
4	5	0,0207	2,2663	8,7248	121,3182	9	5	0,0174	2,2631	8,7215	121,3149
5	1	0,0154	2,2611	8,7195	121,3130	10	1	0,0207	2,2663	8,7248	121,3182
5	2	0,0154	2,2611	8,7195	121,3130	10	2	0,0207	2,2663	8,7248	121,3182
5	3	0,0154	2,2611	8,7195	121,3130	10	3	0,0207	2,2663	8,7248	121,3182
5	4	0,0152	2,2609	8,7193	121,2929	10	4	0,0207	2,2663	8,7248	121,3182
5	5	0,0154	2,2611	8,7195	121,3129	10	5	---	---	---	---

Table B.4: *Estimates of the individual covariance matrices* Ω_i *in Model I.*

rat		k_{12}	k_{21}	k_{el}	y_0
1	k_{12}	1,1228	0,0117	0,0117	0,0117
	k_{21}	0,0117	1,1228	0,0117	0,0117
	k_{el}	0,0117	0,0117	1,1229	0,0117
	y_0	0,0117	0,0117	0,0117	1,1228
2		1,1403	0,0292	0,0293	0,0292
		0,0292	1,1403	0,0292	0,0292
		0,0293	0,0292	1,1404	0,0292
		0,0292	0,0292	0,0292	1,1403
3		1,1153	0,0042	0,0042	0,0042
		0,0042	1,1153	0,0042	0,0042
		0,0042	0,0042	1,1153	0,0042
		0,0042	0,0042	0,0042	1,1153
4		1,1140	0,0029	0,0029	0,0029
		0,0029	1,1140	0,0029	0,0029
		0,0029	0,0029	1,1140	0,0029
		0,0029	0,0029	0,0029	1,1140
5		1,1212	0,0101	0,0101	0,0098
		0,0101	1,1212	0,0101	0,0098
		0,0101	0,0101	1,1213	0,0098
		0,0098	0,0098	0,0098	1,1206
6		1,1233	0,0122	0,0123	0,0122
		0,0122	1,1233	0,0123	0,0122
		0,0123	0,0123	1,1234	0,0123
		0,0122	0,0122	0,0123	1,1233
7		1,1172	0,0061	0,0061	0,0061
		0,0061	1,1172	0,0061	0,0061
		0,0061	0,0061	1,1173	0,0061
		0,0061	0,0061	0,0061	1,1172
8		1,1128	0,0016	0,0017	0,0016
		0,0016	1,1128	0,0017	0,0016
		0,0017	0,0017	1,1128	0,0017
		0,0016	0,0016	0,0017	1,1128
9		1,1127	0,0016	0,0016	0,0016
		0,0016	1,1127	0,0016	0,0016
		0,0016	0,0016	1,1127	0,0016
		0,0016	0,0016	0,0016	1,1128
10		1,1115	0,0004	0,0004	0,0004
		0,0004	1,1115	0,0004	0,0004
		0,0004	0,0004	1,1115	0,0004
		0,0004	0,0004	0,0004	1,1115

Table B.5: *Estimates of the population covariance matrix* Σ *in Model I.*

$\hat{\Sigma}$	k_{12}	k_{21}	k_{el}	y_0
k_{12}	0,2939	0,2939	0,2940	0,2939
k_{21}	0,2939	0,2939	0,2940	0,2939
k_{el}	0,2940	0,2940	0,2940	0,2940
y_0	0,2939	0,2939	0,2940	0,2939