Interindividual and interoccasion variability of toxicokinetic parameters in population models

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Abstract

The determination of toxicokinetic parameters is an essential component in the risk assessment of potential harmful chemicals. It's a first step to analyse the processes which are involved in the development of DNA adducts and might therefore lead to the development of cancer.

The complete research depends on investigations with animals *in vivo* and *in vitro,* so that a critical step is the extrapolation from experimental animals to the human organism. Besides the investigation of the interspecific differences, the intraspecific and the interoccasion variability have to be analysed to avoid serious errors in the determination of the human risk.

The aim of extrapolation from one species to an other requires a characterisation of the interesting processes which is valid for the whole species, i.e. population mean parameters instead of sets of parameters for different individuals, occasions and concentrations of the interesting chemical.

The theory of hierarchical models, basically the work of Racine-Poon et al. (1985, 1986, 1990), provides a procedure, which incorporates both, modelling of the variability structure and reduction of complexity in terms of estimates of population mean parameter vectors.

This paper presents part of a strategy to determinate the processes of uptake, elimination, and metabolism of the gas ethylene, which is a natural body constituent and an important industrial chemical.

Key Words: EM algorithm, two-compartment model, population model, Bayes estimates, toxicokinetics, nonlinear hierarchical model

1. Introduction

The determination of toxicokinetic parameters is an essential component in the risk assessment of potential harmful chemicals. Most chemical carcinogens are transformed into a chemical active form, its *metabolite,* that is able to interact with many of the cellular macromolecules such as DNA, RNA, and protein, and might therefore lead to the development of cancer. It seems that the relationship between applied dose and tumor response is usually nonlinear. This nonlinearity is supposed to be due to the kinetic processes involved in the development of

DNA adducts (Hoel et al., 1983). So a first step to assess the risk of a xenobiotic is to investigate the kinetic processes of uptake, elimination, and metabolism.

As the complete research depends on experiments with animals *in vivo* and *in vitro*, a critical step is the extrapolation from the risk observed in the experimental animals to the risk associated with the human organism. Besides the examination of the interspecific differences, the variability structure within the observed species has to be analysed to avoid serious errors in the assessment of the human risk. Studies of the intraspecific variation of toxicokinetic parameters require two main sources of variability to be accounted for: the *interindividual* variability, and the *interoccasion* variability, i. e. the variation in the individual parameters at repeated examinations. The latter appears to be the primary source of toxicokinetic variability.

The aim of extrapolation from one species to an other demands a characterisation of the interesting processes which is valid for the whole species, i.e. population mean parameters. Thus, analysing kinetic processes, we have to consider both, the modelling of the variability structure and the evaluation of a population model.

Such a procedure is provided by the theory of hierarchical models, as given by Racine-Poon et al. (1985, 1986, 1990). In this paper we present part of a strategy to investigate the processes of uptake, elimination, and metabolism of the gas ethylene applying a two-compartment model, and, moreover, incorporating prior information out of preceding experiments, which is often available in toxicokinetic research. The actual study, performed at the *Institut für Arbeitsphysiologie an der Universität Dortmund*, combines a repeated measurement design with the investigation of a larger range of concentrations of ethylene.

2. Project

The aim of this investigation is to determinate the population mean kinetic parameters of uptake, elimination, and metabolism of the chemical ethylene and to quantify the variability due to interindividual and interoccasion differences.

Ethylene is an important industrial chemical, which is present in environmental and industrial atmospheres. Ethylene is also a vegetable hormone involved in the process of ripening and, moreover, it is a natural body constituent of mammalian organisms. Preceding studies reveal, that ethylene is metabolised to ethylene oxide, which is a directly alkylating and genotoxic agent. Ethylene oxide is carcinogenic in experimental animals, its human carcinogenicity is still debated (Bolt, 1998).

Former inhalation experiments with ethylene indicate that the metabolism can be well approximated by first order kinetics at concentrations below 800 ppm. At higher concentrations the metabolism becomes more and more saturated (Bolt & Filser, 1987).

Experimental design

Two different groups of experiments were performed, each of which with 10 male Sprague-Dawley rats. The animals had an average weight of 300 g at the beginning of the investigation. Both groups of experiments were carried out using the "closed chamber technique" as reviewed by Filser (1992), which allows investigations of kinetics of volatile chemicals *in vivo*. This technique is based on a closed inhalation chamber where during the exposure period the atmospheric concentrations of the substance, in this case ethylene, are measured.

In the inhalation chamber, the experimental animals are exposed to the gas or vapour of interest. The exhaled CO_2 is absorbed by soda lime, and its volume is replaced by pure oxygen. At the beginning of each experiment, the test material (here: ethylene) is injected into the chamber. In the course of time, the change of the atmospheric concentration within the chamber is measured by gas-chromatographic means. Due to the way of application, the actual concentration in the inhalation chamber at the beginning of each experiment, i. e. at zero time, is not exactly known.

The experiments of the first group (group A) had the following design:

Each of the ten rats was exposed to a concentration of about 100 ppm ethylene for a time period of about 8 hours. In that time the concentration of ethylene in the atmosphere was measured at several time points. This procedure was repeated four times with the same initial concentration of about 100 ppm ethylene, so that we finally received five short time series per animal observed under the same conditions (cf. Fig. 1).

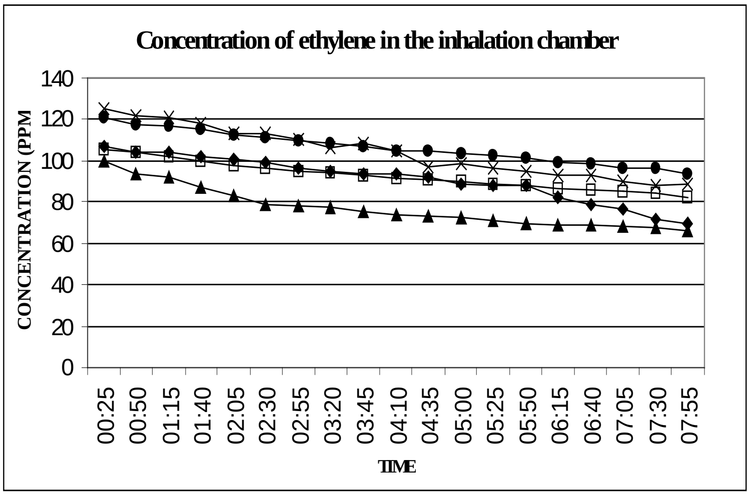

Figure 1. *Observations of one single Sprague-Dawley rat at five occasions (initial concentrations about 100 ppm); time in hours since application of ethylene.*

The experiments of the second group (group B) were realised with 10 other rats in a similar way. For each animal we also observed five concentration-time curves but at five different initial concentrations of 20 ppm, 50 ppm, 100 ppm, 200 ppm and 500 ppm ethylene (cf. Fig. 2).

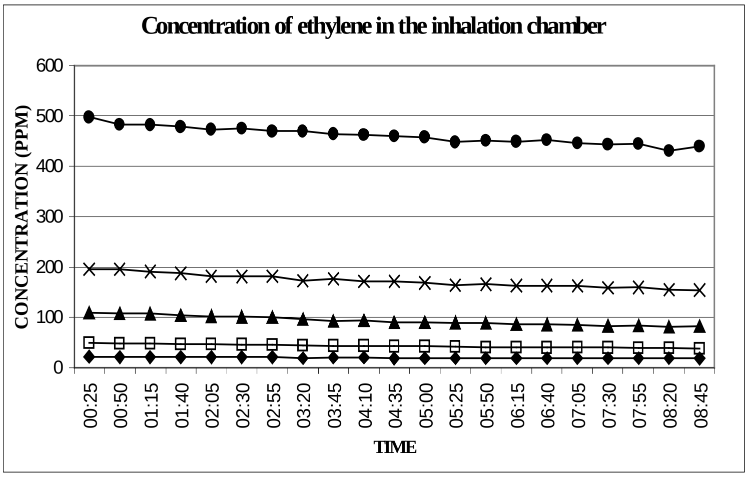

Figure 2. *Observations of one single Sprague-Dawley rat at five initial concentrations of about 20, 50, 100, 200, and 500 ppm; time in hours since application of ethylene.*

The applied doses of ethylene were below the point of saturation of ethylene of about 800 ppm.

3. Statistical models and methods

3.1 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 represented by two compartments, the first C_1 being 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 transits from one compartment to the other through a theoretical interface. During this process, a portion of the xenobiotic within the organism at any stage is eliminated by metabolic processes, and another portion is exhaled (cf. Fig. 3).

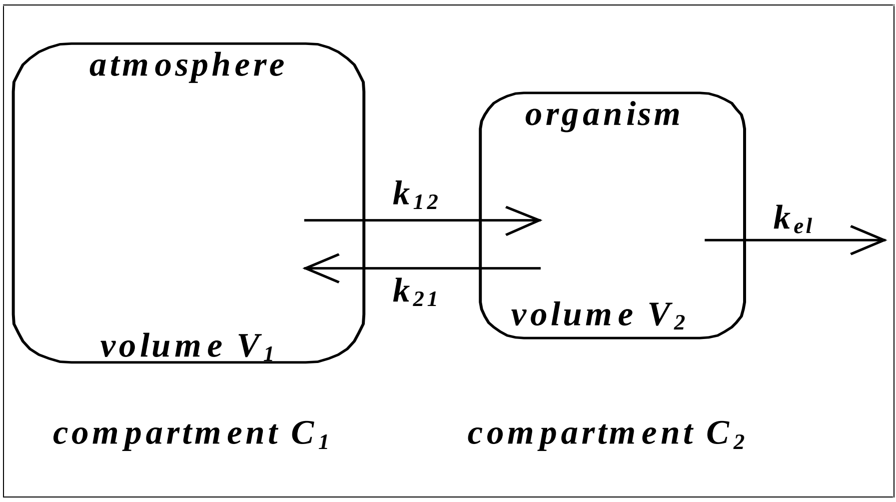

Figure 3. *Two-compartment block model in the case of metabolic turnover*

In the case of ethylene this substance is eliminated by the production of its reactive metabolite ethylene oxide which leads to the alkylation of DNA. Special interest is given to the kinetics governing these processes.

This paper concentrates on overall first order kinetic processes. Preceding investigations indicated that the range of initial concentrations that we used here was below the point of saturation of ethylene at about 800 ppm, so that the processes may be approximated well by first order kinetics (Bolt & Filser, 1987).

Moreover Becka (1998) showed that first order kinetics can be used as good approximations for nonlinear kinetic processes, Michaelis-Menten kinetics, for instance, if the observed maximum concentrations don't exceed the point of saturation.

Let $y_l(t)$ 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} for the uptake, k_{21} for the exhalation, and k_{el} for the metabolic elimination (cf. Fig. 3). Thus the two-compartment model can be described by a system of linear differential equations (Becka et al., 1993):

$$V_1 \cdot \frac{\partial y_1(t)}{\partial t} = -k_{12} \cdot V_1 \cdot y_1(t) + k_{21} \cdot V_2 \cdot y_2(t) \tag{1.1}$$

$$V_2 \cdot \frac{\partial y_2(t)}{\partial t} = +k_{12} \cdot V_1 \cdot y_1(t) - (k_{21} + k_{el}) \cdot V_2 \cdot y_2(t) \tag{1.2}$$

or rather

$$\dot{y}(t) := \begin{pmatrix} \dfrac{\partial y_1(t)}{\partial t} \\ \dfrac{\partial y_2(t)}{\partial t} \end{pmatrix} = A(\vec{k}) \cdot y(t) \tag{1.3}$$

with the notation

$$A(\varphi) := \begin{pmatrix} -k_{12} & \alpha_2 \cdot k_{21} \\ \alpha_1 \cdot k_{12} & -(k_{21} + k_{el}) \end{pmatrix}, \tag{1.4}$$

$\varphi = (k_{12}, k_{21}, k_{el})^T$, $\alpha_1 := V_1/V_2$, $\alpha_2 := V_2/V_1$, and $y(t) := (y_1(t), y_2(t))^T$.

The solution is given by

$$y_1(t) = y_1(0) \cdot \left\{ \frac{(k_{12} + \lambda_1)\exp\{\lambda_2 t\} - (k_{21} + \lambda_2)\exp\{\lambda_1 t\}}{(\lambda_1 - \lambda_2)} \right\}, \tag{1.5}$$

and

$$y_2(t) = y_1(0) \cdot \left\{ \frac{(k_{12} + \lambda_1)(k_{12} + \lambda_2)}{(\lambda_1 - \lambda_2)\alpha_2 k_{21}} \cdot [\exp\{\lambda_2 t\} - \exp\{\lambda_1 t\}] \right\} \tag{1.6}$$

where $\lambda_{1,2} = \frac{1}{2}\left\{-\left(k_{12} + k_{21} + k_{el}\right) \pm \sqrt{\left(k_{12} + k_{21} + k_{el}\right)^2 - 4k_{12}k_{el}}\right\}$ are the eigenvalues of the matrix $A(\varphi)$ in (1.4) (Urfer & Becka, 1996).

3.2 Population model for interindividual and interoccasion variability

We fit two different nonlinear hierarchical models, each to one group of experiments incorporating the results of the first into the model of the second.

Notation

The observed concentrations of ethylene in the atmosphere of the exposition system (compartment 1) are denoted by y_{ijk}, with i = 1, . . ., 20 the number of the individual rat
(i = 1, . . ., 10 for the animals in group A, i = 11, . . ., 20 for group B),
j = 1, . . ., J the observations at time points t_j and
k = 1, . . ., 5 the number of the experiments.
For group B the numbers k = 1, . . ., 5 of the experiments correspond to the five different initial concentrations of about 20 ppm, 50 ppm, 100 ppm, 200 ppm and 500 ppm. For the experiments of group A the index k serves to distinguish the five concentration-time curves per individual with similar initial concentrations of about 100 ppm. Due to the different experimental designs, the assumptions vary for both models:

<u>Group A:</u> $y_{ijk} = f(\beta_{ik}, t_j) + \varepsilon_{ijk}$, i = 1, . . ., 10, j = 1, . . ., J, k = 1, . . ., 5,

where $f(\beta_{ik}, t_j)$ is a nonlinear function of the individual parameter vector β_{ik} and the time t. The function $f(\beta_{ik}, t_j)$ 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 kinetic parameters, differs from individual to individual and is of dimension p = 4.
The initial concentration in compartment 1 for the ith rat in the kth experiment, $y_{ik}(0)$, is approximately 100 ppm ethylene.

Group B: $y_{ijk} = f(\theta_i, t_{jk}) + \varepsilon_{ijk}$, $i = 11, \ldots, 20, j = 1, \ldots, J, k = 1, \ldots, 5,$

where $f(\theta_i, t_{jk})$ is a nonlinear function of the individual parameter vector θ_i and the time t. The function $f(\theta_i, t_{jk})$ denotes the expected concentration-time curve for the ith individual under different experimental conditions. The parameter vector

$\theta_i = (\varphi_i^T, y_{i1}(0), y_{i2}(0), y_{i3}(0), y_{i4}(0), y_{i5}(0))^T$, with $\varphi_i = (k_{12i}, k_{21i}, k_{eli})^T$, differs from individual to individual and is of dimension $p = 8$. The initial concentrations are about 20 ppm, 50 ppm, 100 ppm, 200 ppm and 500 ppm.

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. Note that, in contrast to group B, for group A these five initial concentrations per animal are of the same magnitude.

For both groups of experiments we observe individual responses and get information about the individual parameters φ_{ik} and φ_i, respectively. Our main interest are not the individual processes with individual parameters but a population mean process with parameters $\varphi = (k_{12}, k_{21}, k_{el})^T$, which underlies the different individual processes. The individual parameter vectors φ_{ik} and φ_i , respectively, may be regarded as to vary at random across a population mean parameter vector φ. Additionally we suppose that the variances of the observed concentration-time curves differ from individual to individual. The experiments of group A provide information about the individual covariance structure as we have five observation per time point and animal due to the same experimental conditions.

The idea is now to apply nonlinear hierarchical models to both data sets using a Bayesian approach as suggested by Racine-Poon (1985). We obtain the information about the intersubject and interoccasion variability from group A and use the results to estimate the individual and population mean parameters from group B.

3.2.1 Investigation of intersubject and interoccasion variability (Group A)

A Bayesian approach according to Racine-Poon (1985) and Racine-Poon and Smith (1990) is applied to the data of group A, which consists of five concentration-time curves per individual measured under similar experimental conditions. 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 denoted by (◊).

We assume 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: $\quad y_{ijk} \sim N(f(\beta_{ik},t_j),\ \tau_{ik}^2) \quad i = 1, \ldots, 10,\ j = 1, \ldots, J$ and $k = 1, \ldots, 5$,

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: $\quad \beta_{ik} \sim N(\beta_i, \Omega_i)\ i = 1, \ldots, 10$ and $k = 1, \ldots, 5$,

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

given β, Σ^A: $\quad \beta_i \sim N(\beta, \Sigma^A) \quad i = 1, \ldots, 10,$

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

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

For unknown variances τ_{ik}^2 and covariance matrices Ω_i and Σ^A Racine-Poon (1985) presents a method to estimate the parameter vectors β_{ik}, β_i and β as well as τ_{ik}^2, Ω_i and Σ^A using a vague prior distribution for τ_{ik}^2. The inverse covariance matrices Ω_i^{-1} and $\Sigma^{A^{-1}}$ are assumed to follow Wishart distributions with degrees of freedom ρ_1 and ρ_2 and matrices R_1 and R_2, respectively, so that $R_1^{-1}/(\rho_1-p-1)$ and $R_2^{-1}/(\rho_2-p-1)$ play the role of prior estimates of Ω_i and Σ^A. If there is only little information about Ω_i^{-1} and $\Sigma^{A^{-1}}$ available, the degrees of freedom ρ_1 and ρ_2 are set equal to the dimension p of the parameter vectors β_{ik} and θ_i. Additionally the variances τ_{ik}^2, $i = 1, \ldots, 10$, $k = 1, \ldots, 5$, Ω_i, $i = 1, \ldots, 10$, Σ^A and β should be independent.

The estimators for both cases, known and unknown variances and covariance matrices, are given by Heiser (1997) for observations y_{ij} of time series or repeated measures and will be adapted for the further analysis. In the latter case an EM algorithm as provided by Dempster et al. (1977) is applied to estimate the parameter vectors as well as the covariance matrices.

Bayes estimates of the parameters in the nonlinear model for group A

We get the Bayesian estimators 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

$$\zeta_{ik} \sim N\,(\beta_{ik},\ \tau_{ik}^2 C_{ik}), \quad i = 1, \ldots, 10,\ k = 1, \ldots, 5.$$

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 $\beta_{ik},\ \tau_{ik}^2$: $\quad \zeta_{ik} \sim N\,(\beta_{ik},\ \tau_{ik}^2 C_{ik}), \quad i = 1, \ldots, 10,\ k = 1, \ldots, 5$

given β_i ,Ω_i: $\quad \beta_{ik} \sim N\,(\beta_i, \Omega_i), \quad i = 1, \ldots, 10,\ k = 1, \ldots, 5$

given β, Σ^A: $\quad \beta_i \sim N\,(\beta, \Sigma^A), \quad i = 1, \ldots, 10$

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

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

$$\tau_{ik}^{-2}C_{ik}^{-1} = E\left[-\frac{\partial^2}{\partial\beta_{ik}\partial\beta_{ik}^T}\ln L\left(y_{1,1,1},\ldots,y_{10,J,5}\middle|\beta_{1,1},\ldots,\beta_{10,5},\tau_{1,1}^2,\ldots,\tau_{10,5}^2\right)\right] \tag{2.1.1}$$

Using the assumptions made for the observations y_{ijk} (2.1.1) can be calculated as follows:

$$C_{ik}^{-1} = \begin{pmatrix} \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik1}}\right)^2 & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik1}}\right)\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik2}}\right) & \cdots & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik1}}\right)\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik4}}\right) \\ \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik1}}\right)\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik2}}\right) & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik2}}\right)^2 & \cdots & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik2}}\right)\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik4}}\right) \\ \vdots & \vdots & \ddots & \vdots \\ \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik1}}\right)\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik4}}\right) & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik2}}\right)\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik4}}\right) & \cdots & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik},t_j)}{\partial\beta_{ik4}}\right)^2 \end{pmatrix} \tag{2.1.2}$$

The vectors of parameters β_{ik} in (2.1.2) are substituted by their maximum likelihood estimates ζ_{ik}, $i = 1, \ldots, 10$, $k = 1, \ldots, 5$.

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 Section 3.1 the transport processes in an open two-compartment model are characterised by the differential equations (1.1) and (1.2).

Setting $y_{ik}(t_j)$ equal to $f(\beta_{ik}, t_j)$ in (1.5) yields

$$f(\beta_{ik},t_j) = y_{ik}(0)\cdot\left\{\frac{(k_{12ik}+\lambda_{1ik})\exp\{\lambda_{2ik}t_j\} - (k_{21ik}+\lambda_{2ik})\exp\{\lambda_{1ik}t_j\}}{(\lambda_{1ik}-\lambda_{2ik})}\right\}, \tag{2.1.3}$$

where λ_{1ik} and λ_{2ik} are the eigenvalues of the matrix $A(\varphi_{ik})$, corresponding to the matrix $A(\varphi)$ in (1.4). The eigenvalues only depend on the kinetic parameters k_{12ik}, k_{21ik}, and k_{elik} :

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

with $\lambda_{2ik} < \lambda_{1ik} < 0$.

The $f(\beta_{ik}, t_j)$ are nonlinear functions that can be divided into the initial concentration $y_{ik}(0)$ and a nonlinear function $g(\varphi_{ik}, t_j)$, which does only depend on the individual kinetic parameters k_{12ik}, k_{21ik}, and k_{elik}:

$$f(\beta_{ik}, t_j) = y_{ik}(0) \cdot g(\varphi_{ik}\ t_j) \tag{2.1.4}$$

with $g(\varphi_{ik}, t_j) = \dfrac{(k_{12ik} + \lambda_{1ik})\exp\{\lambda_{2ik} t_j\} - (k_{21ik} + \lambda_{2i})\exp\{\lambda_{1ik} t_j\}}{(\lambda_{1ik} - \lambda_{2ik})}$.

This notation simplifies the calculation of the Fisher Information matrix (2.1.1).

Given the nonlinear hierarchical model (◊) with $f(\beta_{ik}, t_j)$ as specified in (2.1.3), the sums over the index j in (2.1.2), which involve the partial derivatives with respect to the $y_{ik}(0)$, can be simplified to

$$\sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial y_{ik}(0)}\right)^2 = \sum_{j=1}^{J}\left(\frac{\partial y_{ik}(0)\cdot g(\varphi_{ik}, t_j)}{\partial y_{ik}(0)}\right)^2 = \sum_{j=1}^{J}\left(g(\varphi_{ik}, t_j)\right)^2 \tag{2.1.5}$$

and

$$\begin{aligned}\sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{*ik}}\right)\cdot\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial y_{ik}(0)}\right) &= \sum_{j=1}^{J}\left(\frac{\partial y_{ik}(0) g(\varphi_{ik}, t_j)}{\partial k_{*ik}}\right)\cdot\left(\frac{\partial y_{ik}(0) g(\varphi_{ik}, t_j)}{\partial y_{ik}(0)}\right)\\ &= \sum_{j=1}^{J}\left(\frac{\partial y_{ik}(0) g(\varphi_{ik}, t_j)}{\partial k_{*ik}}\right)\cdot g(\varphi_{ik}, t_j)\end{aligned} \tag{2.1.6}$$

where $* = 12, 21, el$ denotes the indices of the kinetic parameters.

Inserting these sums into (2.1.2) this yields

$$C_{ik}^{-1} = \begin{pmatrix} \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{12ik}}\right)^2 & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{12ik}}\right)\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{21ik}}\right) & \cdots & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{12ik}}\right)\cdot g(\varphi_{ik}, t_j) \\ \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{12ik}}\right)\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{21ik}}\right) & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{21ik}}\right)^2 & \cdots & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{21ik}}\right)\cdot g(\varphi_{ik}, t_j) \\ \vdots & \vdots & \ddots & \vdots \\ \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{12ik}}\right)\cdot g(\varphi_{ik}, t_j) & \sum_{j=1}^{J}\left(\frac{\partial f(\beta_{ik}, t_j)}{\partial k_{21ik}}\right)\cdot g(\varphi_{ik}, t_j) & \cdots & \sum_{j=1}^{J}\left(g(\varphi_{ik}, t_j)\right)^2 \end{pmatrix}$$

(2.1.7)

The sums of first partial derivatives of $f(\beta_{ik}, t_j)$ are given by Becka (1994) as follows

$$
\begin{aligned}
&\frac{\partial\left[f(\beta_{ik},t_j)\right]}{\partial k_{12ik}} = \frac{\partial\left[y_{ik}(0)\cdot g(\varphi_{ik},t_j)\right]}{\partial k_{12ik}} = \\
&y_{ik}(0)\cdot\left\{\left[(1+\Psi_{ik})\cdot t_j\cdot\frac{\partial\lambda_{2ik}}{\partial k_{12ik}}+\frac{\partial\Psi_{ik}}{\partial k_{12ik}}\right]\cdot e^{\lambda_{2ik}\cdot t_j}-\left[\frac{\partial\Psi_{ik}}{\partial k_{12ik}}+\Psi_{ik}\cdot t_j\cdot\frac{\partial\lambda_{1ik}}{\partial k_{12ik}}\right]\cdot e^{\lambda_{1ik}\cdot t_j}\right\}
\end{aligned}
\tag{2.1.8}
$$

with $\Psi_{ik} := \dfrac{(k_{12ik}+\lambda_{2ik})}{(\lambda_{1ik}-\lambda_{2ik})}$,

$$\frac{\partial\lambda_{1ik}}{\partial k_{12ik}} = \frac{1}{2}\left\{\frac{(k_{12ik}+k_{21ik}-k_{elik})}{\sqrt{(k_{12ik}+k_{21ik}+k_{elik})^2-4\cdot k_{12ik}\cdot k_{elik}}}-1\right\},$$

$$\frac{\partial\lambda_{2ik}}{\partial k_{12ik}} = -\frac{1}{2}\left\{\frac{(k_{12ik}+k_{21ik}-k_{elik})}{\sqrt{(k_{12ik}+k_{21ik}+k_{elik})^2-4\cdot k_{12ik}\cdot k_{elik}}}+1\right\}, \text{ and}$$

$$\frac{\partial\Psi_{ik}}{\partial k_{12ik}} = (\lambda_{1ik}-\lambda_{2ik})^{-2}\cdot\left[(\lambda_{1ik}-\lambda_{2ik})\cdot\left(1+\frac{\partial\lambda_{2ik}}{\partial k_{12ik}}\right)-(k_{12ik}-\lambda_{2ik})\cdot\left(\frac{\partial\lambda_{1ik}}{\partial k_{12ik}}-\frac{\partial\lambda_{2ik}}{\partial k_{12ik}}\right)\right],$$

furthermore

$$
\begin{aligned}
&\frac{\partial\left[f(\beta_{ik},t_j)\right]}{\partial k_{21ik}} = \frac{\partial\left[y_{ik}(0)\cdot g(\varphi_{ik},t_j)\right]}{\partial k_{21ik}} = \\
&y_{ik}(0)\cdot\left\{\left[(1+\Psi_{ik})\cdot t_j\cdot\frac{\partial\lambda_{2ik}}{\partial k_{21ik}}+\frac{\partial\Psi_{ik}}{\partial k_{21ik}}\right]\cdot e^{\lambda_{2ik}\cdot t_j}-\left[\frac{\partial\Psi_{ik}}{\partial k_{21ik}}+\Psi_{ik}\cdot t_j\cdot\frac{\partial\lambda_{1ik}}{\partial k_{21ik}}\right]\cdot e^{\lambda_{1ik}\cdot t_j}\right\}
\end{aligned}
\tag{2.1.9}
$$

with $\dfrac{\partial\lambda_{1ik}}{\partial k_{21ik}} = \dfrac{1}{2}\left\{\dfrac{(k_{12ik}+k_{21ik}+k_{elik})}{\sqrt{(k_{12ik}+k_{21ik}+k_{elik})^2-4\cdot k_{12ik}\cdot k_{elik}}}-1\right\}$,

$$\frac{\partial\lambda_{2ik}}{\partial k_{12ik}} = -\frac{1}{2}\left\{\frac{(k_{12ik}+k_{21ik}+k_{elik})}{\sqrt{(k_{12ik}+k_{21ik}+k_{elik})^2-4\cdot k_{12ik}\cdot k_{elik}}}+1\right\}, \text{ and}$$

$$\frac{\partial\Psi_{ik}}{\partial k_{12ik}} = (\lambda_{1ik}-\lambda_{2ik})^{-2}\cdot\left[(\lambda_{1ik}-\lambda_{2ik})\cdot\left(\frac{\partial\lambda_{2ik}}{\partial k_{21ik}}\right)-(k_{12ik}-\lambda_{2ik})\cdot\left(\frac{\partial\lambda_{1ik}}{\partial k_{21ik}}-\frac{\partial\lambda_{2ik}}{\partial k_{21ik}}\right)\right],$$

and finally

$$\frac{\partial\left[\ f\left(\beta_{ik},t_j\right)\ \right]}{\partial k_{elik}}=\frac{\partial\left[\ y_{ik}(0)\cdot g\left(\varphi_{ik},t_j\right)\ \right]}{\partial k_{elik}}=$$

$$y_{ik}(0)\cdot\left\{\left[\left(1+\Psi_{ik}\right)\cdot t_j\cdot\frac{\partial\lambda_{2ik}}{\partial k_{elik}}+\frac{\partial\Psi_{ik}}{\partial k_{elik}}\right]\cdot e^{\lambda_{2ik}\cdot t_j}-\left[\frac{\partial\Psi_{ik}}{\partial k_{elik}}+\Psi_{ik}\cdot t_j\cdot\frac{\partial\lambda_{1ik}}{\partial k_{elik}}\right]\cdot e^{\lambda_{1ik}\cdot t_j}\right\} \tag{2.1.10}$$

with $\frac{\partial\lambda_{1ik}}{\partial k_{elik}}=\frac{1}{2}\left\{\frac{\left(-k_{12ik}+k_{21ik}+k_{elik}\right)}{\sqrt{\left(k_{12ik}+k_{21ik}+k_{elik}\right)^2-4\cdot k_{12ik}\cdot k_{elik}}}-1\right\}$,

$\frac{\partial\lambda_{2ik}}{\partial k_{elik}}=-\frac{1}{2}\left\{\frac{\left(-k_{12ik}+k_{21ik}+k_{elik}\right)}{\sqrt{\left(k_{12ik}+k_{21ik}+k_{elik}\right)^2-4\cdot k_{12ik}\cdot k_{elik}}}+1\right\}$, and

$$\frac{\partial\Psi_{ik}}{\partial k_{elik}}=\left(\lambda_{1ik}-\lambda_{2ik}\right)^{-2}\cdot\left[\left(\lambda_{1ik}-\lambda_{2ik}\right)\cdot\left(\frac{\partial\lambda_{2ik}}{\partial k_{elik}}\right)-\left(k_{12ik}-\lambda_{2ik}\right)\cdot\left(\frac{\partial\lambda_{1ik}}{\partial k_{elik}}-\frac{\partial\lambda_{2ik}}{\partial k_{elik}}\right)\right].$$

(i) Estimators in the case of known covariance matrices

In the case of known variances τ_{ik}^2, and covariance matrices Ω_i and Σ^A the posterior distribution of β, given ζ_{ik} , τ_{ik}^2, Ω_i, and Σ^A, i = 1, . . ., 10, k = 1, . . ., 5 is p-variate normal, p = 4, with mean β^* and covariance matrix D, where

$$\beta^*=\left[\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\tau_{ik}^2C_{ik}+\Omega_i+\Sigma^A\right)^{-1}\right]^{-1}\cdot\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\tau_{ik}^2C_{ik}+\Omega_i+\Sigma^A\right)^{-1}\zeta_{ik}=Dd\ , \tag{2.1.11}$$

with $D^{-1}=\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\tau_{ik}^2C_{ik}+\Omega_i+\Sigma^A\right)^{-1}$ and $d=\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\tau_{ik}^2C_{ik}+\Omega_i+\Sigma^A\right)^{-1}\zeta_{ik}$ is the Bayes estimator of the population mean parameter vector β.

The Bayes estimate β^* is normally distributed (Heiser, 1997)

$\beta^* \sim N_4(\beta, D)$.

The individual kinetic processes are characterised by an individual mean parameter vector β_i and experiment specific parameter vectors β_{ik}..

The posterior distribution of β_i, given $\zeta_{1,1}, \ldots, \zeta_{10,5}$, β and Σ^A, are independent p-variate normals, $p = 4$, with means β_i^*, $i = 1, \ldots, 10$, and covariance matrices D_i,, where

$$\beta_i^* = \left[\left[\sum_{k=1}^{5}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1}\right] + \Sigma^{A^{-1}}\right]^{-1} \cdot \left[\left(\sum_{k=1}^{5}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1} \cdot \zeta_{ik}\right) + \Sigma^{A^{-1}} \cdot \beta\right] = D_i d_i, \quad (2.1.12)$$

with $D_i^{-1} = \left[\sum_{k=1}^{5}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1}\right] + \Sigma^{A^{-1}}$ and $d_i = \left[\sum_{k=1}^{5}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1} \cdot \zeta_{ik}\right] + \Sigma^{A^{-1}} \cdot \beta$.

Hence we obtain the Bayes estimate β_i^* as given in (2.1.12), with mean

$$E\left(\beta_i^*\right) = D_i \cdot \left[\sum_{k=1}^{5}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1} \cdot \beta_i\right] + \Sigma^{A^{-1}} \cdot \beta \quad (2.1.13)$$

and covariance matrix

$$Cov\left(\beta_i^*\right) = D_i \cdot \left[\sum_{k=1}^{5}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1}\right] \cdot D_i .$$

The posterior distributions for the parameter vectors β_{ik}, $i = 1, \ldots, 10$, $k = 1, \ldots, 5$, given $\zeta_{1,1}, \ldots, \zeta_{10,5}$, β, Ω_i and Σ^A are p-variate normal, $p = 4$, with means β_{ik}^* and covariance matrices D_{ik}. Thus the Bayes estimate β_{ik}^* is given by

$$\beta_{ik}^* = \left[\left(\tau_{ik}^2 C_{ik}\right)^{-1} + \left(\Omega_i + \Sigma^A\right)^{-1}\right]^{-1} \cdot \left[\left(\tau_{ik}^2 C_{ik}\right)^{-1} \cdot \zeta_{ik} + \left(\Omega_i + \Sigma^A\right)^{-1} \cdot \beta\right] = D_{ik} d_{ik} \quad (2.1.14)$$

$i = 1, \ldots, 10$, $k = 1, \ldots, 5$,

with $D_{ik}^{-1} = \left(\tau_{ik}^2 C_{ik}\right)^{-1} + \left(\Omega_i + \Sigma^A\right)^{-1}$ and $d_{ik} = \left(\tau_{ik}^2 C_{ik}\right)^{-1} \cdot \zeta_{ik} + \left(\Omega_i + \Sigma^A\right)^{-1} \cdot \beta$.

The estimators are normally distributed with means

$$E\left(\beta_{ik}^*\right) = D_{ik} \cdot \left[\left(\tau_{ik}^2 C_{ik}\right)^{-1} \cdot \beta_{ik} + \left(\Omega_i + \Sigma^A\right)^{-1} \cdot \beta\right] \quad (2.1.15)$$

and covariance matrices

$$Cov\left(\beta_{ik}^*\right) = D_{ik} \cdot \left[\left(\tau_{ik}^2 C_{ik}\right)^{-1}\right] \cdot D_{ik} .$$

As β will be unknown in the practical application we replace it in (2.1.12) to (2.1.15) by its Bayes estimate β^*.

The previous estimators are based on known covariance matrices. But in fact we only have rather vague knowledge about these covariance matrices and furthermore the aim of our investigation is to gain information about just these covariances, especially with regard to the interoccasion and interindividual variability. So we need a method to estimate both the parameter vectors and the covariance matrices. Such a method is presented in the following section.

(ii) Estimators in the case of unknown covariance matrices

In the case of unknown variances τ_{ik}^2, i = 1, . . ., 10, k = 1, . . ., 5, Racine-Poon and Smith (1990) suggest to replace them 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

$$\hat{\tau}_{ik}^2 = \frac{1}{J} \cdot \sum_{j=1}^{J} \left(y_{ijk} - f\left(\zeta_{ik}, t_j\right)\right)^2 \quad , i = 1, \ldots, 10,\ k = 1, \ldots, 5. \tag{2.1.16}$$

For unknown covariance matrices Racine-Poon and Smith (1990) suggest an EM-type iterative algorithm as proposed by Dempster et al. (1977) to estimate the individual and the population mean parameters as well as the covariance matrices $\Omega_1, \ldots, \Omega_{10}$ and Σ^A. We adapt this algorithm to our four stage model assuming, as already mentioned, that the inverse covariance matrices Ω_i^{-1}, i = 1, . . ., 10, and $\Sigma^{A^{-1}}$ follow Wishart distributions with degrees of freedom ρ_1 and ρ_2 and matrices R_1 and R_2, respectively. Thus $R_1^{-1}/(\rho_1 - p-1)$ and $R_2^{-1}/(\rho_2 - p-1)$ play the role of prior estimates of Ω_i and Σ^A and the joint posterior density for $\beta_{1,1}, \ldots, \beta_{10,5}, \beta_1, \ldots, \beta_{10}, \beta$, $\Omega_1^{-1}, \ldots, \Omega_{10}^{-1}$ and $\Sigma^{A^{-1}}$, given $\zeta_{1,1}, \ldots, \zeta_{10,5}$, is proportional to

$$\left(\prod_{i=1}^{10}\prod_{k=1}^{5}\left|\hat{\tau}_{ik}^{2}C_{ik}\right|^{-1/2}\right)\cdot\exp\left\{-\frac{1}{2}\sum_{i=1}^{10}\sum_{k=1}^{5}\frac{1}{\hat{\tau}_{ik}^{2}}\left(\zeta_{ik}-\beta_{ik}\right)^{T}\cdot C_{ik}^{-1}\left(\zeta_{ik}-\beta_{ik}\right)\right\}\cdot$$

$$\left(\prod_{i=1}^{10}\left|\Omega_{i}\right|^{-5/2}\right)\cdot\exp\left\{-\frac{1}{2}\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\beta_{ik}-\beta_{i}\right)^{T}\Omega_{i}^{-1}\left(\beta_{ik}-\beta_{i}\right)\right\}\cdot$$

$$\left|\Sigma^{A}\right|^{-10/2}\cdot\exp\left\{-\frac{1}{2}\sum_{i=1}^{10}\left(\beta_{i}-\beta\right)^{T}\Sigma^{A^{-1}}\left(\beta_{i}-\beta\right)\right\}\cdot \tag{2.1.17}$$

$$\prod_{i=1}^{10}\left|\Omega_{i}\right|^{-1/2(\rho_{1}-p-1)}\cdot\exp\left\{-\frac{1}{2}\sum_{i=1}^{10}tr\left(R_{1}^{-1}\cdot\Omega_{i}^{-1}\right)\right\}\cdot$$

$$\left|\Sigma^{A}\right|^{-1/2(\rho_{2}-p-1)}\cdot\exp\left\{-\frac{1}{2}tr\left(R_{2}^{-1}\cdot\Sigma^{A^{-1}}\right)\right\}$$

Vague knowledge about the inverse covariance matrices Ω_1^{-1}, . . ., Ω_{10}^{-1}, and $\Sigma^{A^{-1}}$ 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, $\beta_{ik}^{(l)}$, $\beta_i^{(l)}$, $\beta^{(l)}$, $\Omega_i^{(l)}$, and $\Sigma^{A(l)}$, i = 1, . . ., 10, k = 1, . . ., 5, by replacing the covariance matrices by their current approximations $\Omega_1^{(l-1)}$, . . ., $\Omega_{10}^{(l-1)}$, and $\Sigma^{A(l-1)}$ in (2.1.11), (2.1.12), and (2.1.14) (E-Step) and subsequent calculation of $\Omega_1^{(l)}$, . . ., $\Omega_{10}^{(l)}$, and $\Sigma^{A(l)}$ as the posterior modes using $\beta_{ik}^{(l)}$, $\beta_i^{(l)}$, and $\beta^{(l)}$, i = 1, . . ., 10, k = 1, . . ., 5 (M-Step).

<u>E-Step</u>

Approximating Ω_1, . . ., Ω_{10}, Σ^A in (2.1.11) by $\Omega_1^{(l)}$, . . ., $\Omega_{10}^{(l)}$, and $\Sigma^{A(l)}$ we obtain

$$\beta^{(l)}=\left[\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\hat{\tau}_{ik}^{2}C_{ik}+\Omega_{i}^{(l-1)}+\Sigma^{A(l-1)}\right)^{-1}\right]^{-1}\cdot\sum_{i=1}^{10}\sum_{k=1}^{5}\left(\hat{\tau}_{ik}^{2}C_{ik}+\Omega_{i}^{(l-1)}+\Sigma^{A(l-1)}\right)^{-1}\zeta_{ik} \tag{2.1.18}$$

Substituting β, Ω_i, and Σ^A in (2.1.12) by $\beta^{(l)}$, $\Omega_i^{(l-1)}$, and $\Sigma^{A(l-1)}$, respectively, yields

$$\beta_{i}^{(l)}=\left[\left[\sum_{k=1}^{5}\left(\hat{\tau}_{ik}^{2}C_{ik}+\Omega_{i}^{(l-1)}\right)^{-1}\right]+\Sigma^{A(l-1)^{-1}}\right]^{-1}\cdot\left[\left(\sum_{k=1}^{5}\left(\hat{\tau}_{ik}^{2}C_{ik}+\Omega_{i}^{(l-1)}\right)^{-1}\cdot\zeta_{ik}\right)+\Sigma^{A(l-1)^{-1}}\cdot\beta^{(l)}\right]$$

(2.1.19)

In the same way we get $\beta_{ik}^{(l)}$ by replacing the unknown parameters by their current estimates in (2.1.14):

$$\beta_{ik}^{(l)} = \left[\left(\hat{\tau}_{ik}^2 C_{ik} \right)^{-1} + \left(\Omega_i^{(l-1)} + \Sigma^{A(l-1)} \right)^{-1} \right]^{-1} \cdot \left[\left(\hat{\tau}_{ik}^2 C_{ik} \right)^{-1} \cdot \zeta_{ik} + \left(\Omega_i^{(l-1)} + \Sigma^{A(l-1)} \right)^{-1} \cdot \beta^{(l)} \right]. \quad (2.1.20)$$

<u>M-Step</u>

Conditioning on $\beta_{ik} = \beta_{ik}^{(l)}$, $\beta_i = \beta_i^{(l)}$ and $\beta = \beta^{(l)}$, i = 1, . . ., 10, k = 1, . . ., 5, the conditional posterior mode of (2.1.17) is given by

$$\Omega_i^{(l)} = \frac{R_1^{-1} + \sum_{k=1}^{5} \left(\beta_{ik}^{(l)} - \beta_i^{(l)} \right) \cdot \left(\beta_{ik}^{(l)} - \beta_i^{(l)} \right)^T}{10 + \rho_1 - p - 1}, \qquad i = 1, \ldots, 10, \text{ and} \qquad (2.1.21)$$

$$\Sigma^{A(l)} = \frac{R_2^{-1} + \sum_{i=1}^{10} \left(\beta_i^{(l)} - \beta^{(l)} \right) \left(\beta_i^{(l)} - \beta^{(l)} \right)^T}{10 + \rho_2 - p - 1} \qquad (2.1.22)$$

Both steps are repeated until $\Omega_1^{(l)}, \ldots, \Omega_{10}^{(l)}$, and $\Sigma^{A(l)}$ 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 $\Omega_1^{(0)}, \ldots, \Omega_{10}^{(0)}$, and $\Sigma^{A(0)}$ are given by

$$\Omega_i^{(0)} = \frac{R_1^{-1} + \sum_{k=1}^{5} \left(\zeta_{ik} - \overline{\zeta}_{i.} \right) \left(\zeta_{ik} - \overline{\zeta}_{i.} \right)^T}{10 + \rho_2 - p - 2}, \qquad i = 1, \ldots, 10$$

$$\Sigma^{A(0)} = \frac{R_2^{-1} + \sum_{i=1}^{10} \left(\overline{\zeta}_{i.} - \overline{\zeta}_{..} \right) \left(\overline{\zeta}_{i.} - \overline{\zeta}_{..} \right)^T}{10 + \rho_2 - p - 3},$$

where $\overline{\zeta}_{i.} = \frac{1}{5} \sum_{k=1}^{5} \zeta_{ik}$ and $\overline{\zeta}_{..} = \frac{1}{10} \sum_{i=1}^{10} \overline{\zeta}_{i.} = \frac{1}{50} \sum_{i=1}^{10} \sum_{k=1}^{5} \zeta_{ik}$.

3.2.2 Population model for group B

For the experiments of group B, where the animals will be investigated under different initial conditions, also a Bayesian approach is applied to model the individual responses and their variation across the population mean response.

We state here a three-stage nonlinear hierarchical model denoted by (∗).

We assume 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 θ_i , σ_i^2: $\quad y_{ijk} \sim N\,(f\,(\theta_i\,,\,t_{jk}\,),\ \sigma_i^2\,)$ $\qquad i = 11, \ldots, 20\ j = 1, \ldots, J,\ k = 1, \ldots, 5,$

with $\theta_i = (\varphi_i^T\,,\, y_{i1}(0), y_{i2}(0), y_{i3}(0), y_{i4}(0), y_{i5}(0))^T$, and $\varphi_i = (k_{12}, k_{21}, k_{el})^T$,

given θ, Σ^B: $\quad \theta_i \sim N\,(\theta, \Sigma^B) \qquad i = 11, \ldots, 20,$

with $\theta = (\varphi^T, y_1(0), y_2(0), y_3(0), y_4(0), y_5(0))^T$, and $\varphi = (k_{12i}, k_{21i}, k_{eli})^T$,

$p(\theta) \propto 1 \qquad \forall\ \theta \in IR^8.$

The Bayes estimate of Σ^A can be used as the corresponding components of the prior estimate $\hat{\Sigma}^B = R^{-1}/(\rho - p - 1)$ and hence as prior information of Σ^B. Another possibility is to use Σ^{A*} as the corresponding components of the starting value $\Sigma^{B(0)}$ and include further information from preliminary examinations.

Estimation of the parameters of the nonlinear model for group B

The nonlinear hierarchical model (∗) is transformed into a linear one corresponding to the procedure in Section 3.2.. So the observations y_{ijk} are replaced by the maximum likelihood estimates $\hat{\theta}_i = \zeta_i$ with

$\zeta_i \sim N\,(\theta_i\,,\ \sigma_i^2\, C_i)\,, \qquad i = 11, \ldots, 20.$

Hence we obtain the linear hierarchical model (∗∗):

given θ_i , σ_i^2 : $\zeta_i \sim N(\theta_i , \sigma_i^2 C_i)$, $i = 11, \ldots, 20$

given θ, Σ^B: $\theta_i \sim N(\theta, \Sigma^B)$, $i = 11, \ldots, 20$

$p(\theta) \propto 1$, $\forall\ \theta \in IR^8$,

where the Fisher Information matrix

$\sigma_i^{-2} C_i^{-1} = E\left[-\dfrac{\partial^2}{\partial\theta_i \partial\theta_i^T} \ln L\left(y_{11,1,1},\ldots,y_{20,J,5} \middle| \theta_{11},\ldots,\theta_{20},\sigma_{11}^2,\ldots,\sigma_{20}^2\right)\right]$ is given by

$$\sigma_i^{-2} C_i^{-1} = \begin{pmatrix} \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i1}}\right)^2 & \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i1}}\right)\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i2}}\right) & \cdots & \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i1}}\right)\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i8}}\right) \\ \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i1}}\right)\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i2}}\right) & \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i2}}\right)^2 & \cdots & \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i2}}\right)\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i8}}\right) \\ \vdots & \vdots & \ddots & \vdots \\ \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i1}}\right)\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i8}}\right) & \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i2}}\right)\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i8}}\right) & \cdots & \sum_{j=1}^{J}\sum_{k=1}^{5}\left(\frac{\partial f(\theta_i,t_{jk})}{\partial\theta_{i8}}\right)^2 \end{pmatrix} \tag{2.2.1}$$

The vectors of parameters θ_i in (2.2.1) are substituted by their maximum likelihood estimates ζ_i, $i = 11, \ldots, 20$.

Just like in Section 3.2.1 we assume first order kinetic processes and divide the concentration-time function $f(\theta_i, t_{jk})$ into the initial concentration $y_{ik}(0)$ and the nonlinear function $g(\varphi_i, t_{jk})$, which does not further depend on the different experimental conditions.

$$\begin{aligned} f(\theta_i, t_{jk}) &= y_{ik}(0) \cdot \left\{ \frac{(k_{12i} + \lambda_{1i})\exp\{\lambda_{2i} t_{jk}\} - (k_{21i} + \lambda_{2i})\exp\{\lambda_{1i} t_{jk}\}}{(\lambda_{1i} - \lambda_{2i})} \right\}, \\ &= y_{ik}(0) \cdot g(\varphi_i, t_{jk}) \end{aligned} \tag{2.2.2}$$

with $\lambda_{1i,2i} = \frac{1}{2}\left\{-(k_{12i} + k_{21i} + k_{eli}) \pm \sqrt{(k_{12i} + k_{21i} + k_{eli})^2 - 4k_{12i}k_{eli}}\right\}$, $\lambda_{2i} < \lambda_{1i} < 0$.

Thus, given the nonlinear hierarchical model (∗) with $f\,(\theta_i\,,\,t_{jk}\,)$ as specified in (2.2.2), the sums over the index k in (2.2.1), which involve the partial derivatives with respect to the $y_{ik}(0)$, can be simplified to

$$\sum_{k=1}^{5}\left(\frac{\partial f\left(\theta_i,t_{jk}\right)}{\partial y_{il}(0)}\right)^2=\sum_{k=1}^{5}\left(\frac{\partial y_{ik}(0)\cdot g\left(\varphi_i,t_{jk}\right)}{\partial y_{il}(0)}\right)^2=\left(g\left(\varphi_i,t_{jl}\right)\right)^2, \tag{2.2.3}$$

$$\begin{aligned}\sum_{k=1}^{5}\left(\frac{\partial f\left(\theta_i,t_{jk}\right)}{\partial k_{12i}}\right)\cdot\left(\frac{\partial f\left(\theta_i,t_{jk}\right)}{\partial y_{il}(0)}\right)&=\sum_{k=1}^{5}\left(\frac{\partial y_{ik}(0)g\left(\varphi_i,t_{jk}\right)}{\partial k_{12i}}\right)\cdot\left(\frac{\partial y_{ik}(0)g\left(\varphi_i,t_{jk}\right)}{\partial y_{il}(0)}\right),\\&=\left(\frac{\partial y_{il}(0)g\left(\varphi_i,t_{jl}\right)}{\partial k_{12i}}\right)\cdot g\left(\varphi_i,t_{jl}\right)\end{aligned} \tag{2.2.4}$$

with l = 1, . . ., 5, and

$$\begin{aligned}\sum_{k=1}^{5}\left(\frac{\partial f\left(\theta_i,t_{jk}\right)}{\partial y_{il}(0)}\right)\cdot\left(\frac{\partial f\left(\theta_i,t_{jk}\right)}{\partial y_{im}(0)}\right)&=\sum_{k=1}^{5}\left(\frac{\partial y_{ik}(0)g\left(\varphi_i,t_{jk}\right)}{\partial y_{il}(0)}\right)\cdot\left(\frac{\partial y_{ik}(0)g\left(\varphi_i,t_{jk}\right)}{\partial y_{im}(0)}\right),\\&=g\left(\varphi_i,t_{jl}\right)\cdot 0+0\cdot g\left(\varphi_i,t_{jm}\right)=0\end{aligned} \tag{2.2.5}$$

with l, m = 1, . . ., 5, $l \neq m$, respectively.

Inserting these sums into (2.2.1) this yields

$$C_i^{-1}=\left(\begin{array}{ccc|ccccc}
c_{i11}^{-1} & c_{i12}^{-1} & c_{i13}^{-1} & \sum_{j=1}^{J}g\left(\varphi_i,t_{j1}\right)\cdot\left(\frac{\partial\left[y_{i1}(0)g\left(\varphi_i,t_{j1}\right)\right]}{\partial k_{12i}}\right) & \cdots & \cdots & \cdots & \sum_{j=1}^{J}g\left(\varphi_i,t_{j5}\right)\cdot\left(\frac{\partial\left[y_{i5}(0)g\left(\varphi_i,t_{j5}\right)\right]}{\partial k_{12i}}\right)\\
* & c_{i22}^{-1} & c_{i23}^{-1} & \sum_{j=1}^{J}g\left(\varphi_i,t_{j1}\right)\cdot\left(\frac{\partial\left[y_{i1}(0)g\left(\varphi_i,t_{j1}\right)\right]}{\partial k_{21i}}\right) & \cdots & \cdots & \cdots & \sum_{j=1}^{J}g\left(\varphi_i,t_{j5}\right)\cdot\left(\frac{\partial\left[y_{i5}(0)g\left(\varphi_i,t_{j5}\right)\right]}{\partial k_{21i}}\right)\\
* & * & c_{i33}^{-1} & \sum_{j=1}^{J}g\left(\varphi_i,t_{j1}\right)\cdot\left(\frac{\partial\left[y_{i1}(0)g\left(\varphi_i,t_{j1}\right)\right]}{\partial k_{eli}}\right) & \cdots & \cdots & \cdots & \sum_{j=1}^{J}g\left(\varphi_i,t_{j5}\right)\cdot\left(\frac{\partial\left[y_{i5}(0)g\left(\varphi_i,t_{j5}\right)\right]}{\partial k_{eli}}\right)\\
\hline
 & * & & \sum_{j=1}^{J}\left[g\left(\varphi_i,t_{j1}\right)\right]^2 & 0 & 0 & 0 & 0\\
 & & & * & \sum_{j=1}^{J}\left[g\left(\varphi_i,t_{j2}\right)\right]^2 & 0 & 0 & 0\\
* & & * & & * & \sum_{j=1}^{J}\left[g\left(\varphi_i,t_{j3}\right)\right]^2 & 0 & 0\\
 & & & * & & * & \sum_{j=1}^{J}\left[g\left(\varphi_i,t_{j4}\right)\right]^2 & 0\\
 & * & & & * & & * & \sum_{j=1}^{J}\left[g\left(\varphi_i,t_{j5}\right)\right]^2
\end{array}\right)$$

(2.2.6)

with $c_{ilm}^{-1} = \sum_{j=1}^{J} \sum_{k=1}^{5} \left(\frac{\partial \left[y_{ik}(0) g\left(\varphi_i, t_{jk}\right) \right]}{\partial k_{li}} \right) \cdot \left(\frac{\partial \left[y_{ik}(0) g\left(\varphi_i, t_{jk}\right) \right]}{\partial k_{mi}} \right)$, where indices l and m correspond to *12*, *21*, and *el*. The partial derivatives are given by (2.1.8) to (2.1.10) substituting φ_{ik} by φ_i.

(i) Estimators in the case of known covariance matrices

In the case of known variances σ_i^2 and covariance matrix Σ^B the posterior distribution of θ, given ζ_i, σ_i^2, and Σ^B, $i = 11, \ldots, 20$, is p-variate normal, $p = 8$, with mean θ^* and covariance matrix D, where

$$\theta^* = \left[\sum_{i=11}^{20} \left(\sigma_i^2 C_i + \Sigma^B \right)^{-1} \right]^{-1} \left[\sum_{i=11}^{20} \left(\sigma_i^2 C_i + \Sigma^B \right)^{-1} \zeta_i \right] = Dd \,, \tag{2.2.7}$$

with $D = \left[\sum_{i=11}^{20} \left(\sigma_i^2 C_i + \Sigma^B \right)^{-1} \right]^{-1}$ and $d = \sum_{i=11}^{20} \left(\sigma_i^2 C_i + \Sigma^B \right)^{-1} \zeta_i$, is the Bayes estimator of the population mean parameter vector θ.

Besides the population parameters we also pay attention on the individual kinetic processes. The posterior distributions of the corresponding parameter vectors $\theta_{11}, \ldots, \theta_{20}$ given $\zeta_{11}, \ldots, \zeta_{20}$, θ and Σ^B are independently p-variate normals, $p = 8$, with means θ_i^*, $i = 11, \ldots, 20$, and covariance matrices D_i, where

$$\theta_i^* = \left[\left(\sigma_i^2 C_i \right)^{-1} + \Sigma^{B^{-1}} \right]^{-1} \cdot \left[\left(\sigma_i^2 C_i \right)^{-1} \zeta_i + \Sigma^{B^{-1}} \theta \right] = D_i d_i \,, \qquad i = 11, \ldots, 20, \tag{2.2.8}$$

with $D_i = \left[\left(\sigma_i^2 C_i \right)^{-1} + \Sigma^{B^{-1}} \right]^{-1}$ and $d_i = \left(\sigma_i^2 C_i \right)^{-1} \zeta_i + \Sigma^{B^{-1}} \theta$.

The Bayes estimate θ_i^* as given in (2.2.8) is normally distributed with mean

$$E\left(\theta_i^*\right) = D_i \left[\left(\sigma_i^2 C_i \right)^{-1} \theta_i + \Sigma^{B^{-1}} \theta \right] \tag{2.2.9}$$

and covariance matrix

$$Cov\left(\theta_i^*\right) = D_i \left(\sigma_i^2 C_i \right)^{-1} D_i \,.$$

Again we have to substitute the unknown population mean parameter vector in (2.2.8) and (2.2.9) by its Bayes estimate θ^*.

(ii) Estimators in the case of unknown covariance matrices

For unknown variances σ_i^2, i = 11, . . ., 20, we assume a vague prior distribution and replace them by a suitable estimate $\hat{\sigma}_i^2$, the posterior mode, for instance. Using the previous assumptions and, furthermore, assuming independently distributed variances σ_i^2, i = 11, . . ., 20, we can approximate the Bayes estimate of σ_i^2 by its maximum likelihood estimate

$$\hat{\sigma}_i^2 = \frac{1}{J \cdot 5} \cdot \sum_{j=1}^{J} \sum_{k=1}^{5} \left(y_{ijk} - f\left(\zeta_i, t_{jk}\right) \right)^2 \quad , i = 11, \ldots, 20. \tag{2.2.10}$$

In the case of unknown covariance matrix Σ^B we suppose that its inverse $\Sigma^{B^{-1}}$ follows a Wishart distribution with degrees of freedom ρ and matrix R. Using the estimated Σ^A as the corresponding components of the prior estimate $\hat{\Sigma}^B = R^{-1}/(\rho - p - 1)$ or of the starting value $\Sigma^{B(0)}$ and incorporating further information from preliminary examinations we estimate the individual and population parameters and the covariance matrix Σ^B by means of the EM algorithm given by Dempster et al. (1977). The joint posterior density of θ_{11}, . . ., θ_{20}, θ, and , $\Sigma^{B^{-1}}$, given ζ_{11}, . . ., ζ_{20}, is proportional to

$$\begin{aligned} &\prod_{i=11}^{20} \left| \hat{\sigma}_i^2 C_i \right|^{-1/2} \cdot \exp\left[-\frac{1}{2} \sum_{i=11}^{20} \frac{1}{\hat{\sigma}_i^2} (\zeta_i - \theta_i)^T C_i^{-1} (\zeta_i - \theta_i) \right] \cdot \\ &\left| \Sigma^B \right|^{-(10+\rho-p-1)/2} \cdot \exp\left[-\frac{1}{2} tr\left(\Sigma^{B^{-1}} \left(\sum_{i=11}^{20} (\theta_i - \theta)(\theta_i - \theta)^T + R^{-1} \right) \right) \right] \end{aligned} \quad . \tag{2.2.11}$$

Applying the EM algorithm as described in Section 3.2.1 the lth iteration is given by

E-Step

Approximating Σ^B in (2.2.7) by $\Sigma^{B(l-1)}$ we obtain

$$\theta^{(l)} = \left[\sum_{i=11}^{20} \left(\hat{\sigma}_i^2 C_i + \Sigma^{B(l-1)} \right)^{-1} \right]^{-1} \left[\sum_{i=11}^{20} \left(\hat{\sigma}_i^2 C_i + \Sigma^{B(l-1)} \right)^{-1} \zeta_i \right] \tag{2.2.12}$$

Substituting θ and Σ^B in (2.2.8) by $\theta^{(l)}$ and $\Sigma^{B(l-1)}$, respectively, yields

$$\theta_i^{(l)} = \left[\left(\hat{\sigma}_i^2 C_i \right)^{-1} + \left(\Sigma^{B(l-1)} \right)^{-1} \right]^{-1} \cdot \left[\left(\hat{\sigma}_i^2 C_i \right)^{-1} \zeta_i + \left(\Sigma^{B(l-1)} \right)^{-1} \theta^{(l)} \right] \tag{2.2.13}$$

M-Step

Conditioning on $\theta_i = \theta_i^{(l)}$ and $\theta = \theta^{(l)}$, the posterior mode of (2.2.11) is given by

$$\Sigma^{B(l)} = \frac{R^{-1} + \sum_{i=11}^{20} \left(\theta_i^{(l)} - \theta^{(l)} \right) \left(\theta_i^{(l)} - \theta^{(l)} \right)^T}{10 + \rho - p - 1}. \tag{2.2.14}$$

Both steps are repeated until $\Sigma^{B(l)}$ converges, i. e. the maximum change in the elements of $\Sigma^{B(l)}$ between successive iterations is less than 0.001.

Using the estimated Σ^A to determinate R a reasonable starting value $\Sigma^{B(0)}$ is given by

$$\Sigma^{B(0)} = \frac{R^{-1} + \sum_{i=11}^{20} \left(\zeta_i - \bar{\zeta}_. \right) \left(\zeta_i - \bar{\zeta}_. \right)^T}{10 + \rho - p - 2},$$

where $\bar{\zeta}_. = \frac{1}{10} \sum_{i=11}^{20} \zeta_i$.

4. 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. So before summarising the information provided by experiments within a range of concentrations, like in group B, it is necessary to verify that a first order approximation of the processes is valid. In a further paper we will present a procedure to detect such critical departures from linearity.

Determining the processes involved in the formation of reactive metabolites is just a first step to establish a dose-response relationship for the interesting chemical. The metabolites may be transformed partly into an inactive form. 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.

There exist various attempts 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 out that the parameters depend 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 and Urfer (1998) 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, is applied to estimate regression and covariance parameters.

Our genotoxicological 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) 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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