Estimation of toxicokinetic population parameters in a four-stage hierarchical model

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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.

Fundamental in the extrapolation from one species to another is the characterisation of processes by means of population parameters. Nevertheless, the consideration of individual parameters varying between repeated experiments and different doses is of great importance to obtain a more precise insight into the variability structure of the process so that a valid basis for further research is the final result.

Two nonlinear four-stage hierarchical models for a repeated measurement design and for repeated exposures to different doses 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 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 (Selinski and Urfer, 1998).

Ethylene is an important industrial bulk chemical, which is also present in the environment. In mammalian organisms ethylene is metabolised to ethylene oxide, which is directly alkylating different macromolecules. Ethylene oxide is a physiological body constituent (Bolt, 1996; Bolt et al., 1997) and it's carcinogenic in animal studies; the carcinogenicity in humans is still discussed controversially (Bolt, 1998).

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 based on the approach of Racine-Poon and Smith (1990) are presented; the first one for a repeated measurement design, the second for repeated exposures to different doses. 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).

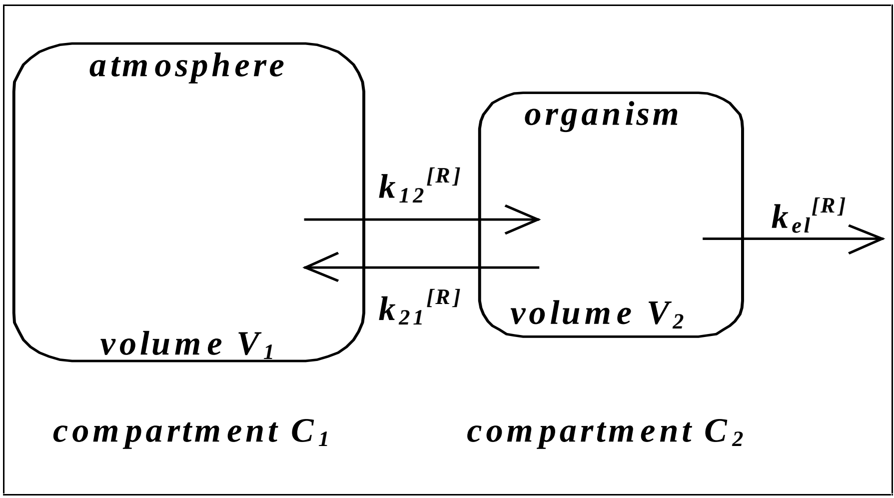

Figure 1. *Two-compartment block model in the case of metabolic turnover*

In the case of ethylene this substance is partly transformed, by hepatic metabolising enzymes (cytochrome P-450) to ethylene oxide (Filser and Bolt, 1983). Ethylene oxide 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). This paper concentrates on overall first order kinetic processes. 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, so that the processes may well be approximated by first order kinetics (Bolt and Filser, 1987).

Moreover, Becka (1998) showed that first order kinetics may also 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 two-compartment model can be described by a system of linear differential equations (Becka et al., 1993):

$$V_1 \cdot \frac{\partial y_1(t)}{\partial t} = -k_{12}^{[R]} \cdot V_1 \cdot y_1(t) + k_{21}^{[R]} \cdot V_2 \cdot y_2(t) \tag{1}$$

$$V_2 \cdot \frac{\partial y_2(t)}{\partial t} = +k_{12}^{[R]} \cdot V_1 \cdot y_1(t) - \left(k_{21}^{[R]} + k_{el}^{[R]}\right) \cdot V_2 \cdot y_2(t) \tag{2}$$

The solution is given by

$$y_1(t) = y(0) \cdot \left\{ \frac{\left(k_{12}^{[R]} + \lambda_1\right)\exp\{\lambda_2 t\} - \left(k_{21}^{[R]} + \lambda_2\right)\exp\{\lambda_1 t\}}{\left(\lambda_1 - \lambda_2\right)} \right\}, \tag{3}$$

and

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

where $\lambda_{1,2} = \frac{1}{2}\left\{ -\left(k_{12}^{[R]} + k_{21}^{[R]} + k_{el}^{[R]}\right) \pm \sqrt{\left(k_{12}^{[R]} + k_{21}^{[R]} + k_{el}^{[R]}\right)^2 - 4k_{12}^{[R]}k_{el}^{[R]}} \right\}$ and $y(0)$ is the initial concentration in compartment 1 (Urfer and Becka, 1996).

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).

According to Filser (1992) the individual rates of uptake $k_{12}^{[R]}$, exhalation $k_{21}^{[R]}$ and metabolic elimination $k_{el}^{[R]}$ are related to the respective rates k_{12}, k_{21} and k_{el} for a standard rat of 1000 ml by

$$k_{12}^{[R]} = k_{12} \cdot v_2^{2/3},$$

$$k_{21}^{[R]} = k_{21} \cdot v_2^{1/3}, \quad \text{and} \tag{5}$$

$$k_{el}^{[R]} = k_{el} \cdot v_2, \qquad \text{where}$$

$v_2 = \left(\frac{1000}{V_2}\right)$ depends on the actual volume of the organism V_2 and the standard volume 1000 ml.

Substituting the real kinetic parameters in the (3) and (4) yields

$$f(\beta,t)=y_1(t)=y(0)\cdot\left\{\frac{\left(k_{12}v_2^{2/3}+\lambda_1\right)\exp\{\lambda_2 t\}-\left(k_{21}v_2^{1/3}+\lambda_2\right)\exp\{\lambda_1 t\}}{(\lambda_1-\lambda_2)}\right\}, \tag{6}$$

and

$$y_2(t)=y(0)\cdot\left\{\frac{\left(k_{12}v_2^{2/3}+\lambda_1\right)\left(k_{12}v_2^{2/3}+\lambda_2\right)}{(\lambda_1-\lambda_2)\alpha_2 k_{21}v_2^{1/3}}\cdot\left[\exp\{\lambda_2 t\}-\exp\{\lambda_1 t\}\right]\right\}, \tag{7}$$

where

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

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 for the experiments of group A and the kth dose for group B.

First of all, we presume that our observations y_{ijk} vary across a nonlinear function $f(\beta_{ik}, t_j)$:

$$y_{ijk} = f(\beta_{ik}, t_j) + \varepsilon_{ijk}, \quad i = 1, \ldots, 10, j = 1, \ldots, J, k = 1, \ldots, 5.$$

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 Hierarchical model for group A

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 $\beta_{ik},\ \tau_{ik}^2$: $\quad y_{ijk} \sim N(\, f(\beta_{ik}, t_j),\ \tau_{ik}^2) \quad i = 1, \ldots, I,\ j = 1, \ldots, J$ 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: $\quad \beta_{ik} \sim N(\beta_i, \Omega_i) \quad i = 1, \ldots, I$ 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: $\quad \beta_i \sim N(\beta, \Sigma) \quad i = 1, \ldots, I$,

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: $\quad \zeta \sim N\,(\theta, V)$,

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: $\quad \theta \sim N\,(Z_2\psi, \Omega)$,

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:

$$\left(\tau_{ik}^2 C_{ik}\right)^{-1} = E\left[-\frac{\partial^2}{\partial\beta_{ik}\partial\beta_{ik}^T}\ln L\left(y_{1,1,1},\ldots,y_{IJK}\middle|\beta_{1,1},\ldots,\beta_{IK},\tau_{1,1}^2,\ldots,\tau_{IK}^2\right)\right] \tag{8}$$

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

$$f\left(\beta_{ik}, t_j\right) = y_{ik}(0)\cdot\left\{\frac{\left(k_{12ik}v_{2ik}^{2/3} + \lambda_{1ik}\right)\exp\{\lambda_{2ik}t_j\} - \left(k_{21ik}v_{2ik}^{1/3} + \lambda_{2ik}\right)\exp\{\lambda_{1ik}t_j\}}{\left(\lambda_{1ik} - \lambda_{2ik}\right)}\right\}, \tag{9}$$

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

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

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

The vectors of parameters β_{ik} in (9) are substituted by their maximum likelihood estimates ζ_{ik}, $i = 1, \ldots, 10$, $k = 1, \ldots, 5$.

Estimates in the case of known covariance matrices

In the case of known variances τ_{ik}^2, and covariance matrices Ω and Λ estimates of the individual and population mean parameters vectors β_{ik}, β_i, *and* β can be calculated following the approach of Lindley and Smith (1972) for linear hierarchical models.

Thus, the posterior distribution of β, given ζ, V, Ω, and Λ is p-variate normal, $p = 4$, with mean β^* and covariance matrix A, where

$$\beta^* = Aa \qquad \text{with} \tag{10}$$

$A^{-1} = Z_3^T Z_2^T \left\{V + \Omega + Z_2 \Lambda Z_2^T\right\}^{-1} Z_2 Z_3$ and $a = Z_3^T Z_2^T \left\{V + \Omega + Z_2 \Lambda Z_2^T\right\}^{-1} \zeta$ is the Bayes estimator of the population mean parameter vector β.

The Bayes estimate β^* is normally distributed with mean β and covariance matrix A.

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 $\beta_1, \ldots, \beta_I$, given ζ, β and Λ, are independent p-variate normals, $p = 4$, with means β_i^*, $i = 1, \ldots, I$, and covariance matrices B_i, where

$$\beta_i^* = \left[\left[\sum_{k=1}^{K}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1}\right] + \Sigma^{-1}\right]^{-1} \cdot \left[\left(\sum_{k=1}^{K}\left(\tau_{ik}^2 C_{ik} + \Omega_i\right)^{-1} \cdot \zeta_{ik}\right) + \Sigma^{-1} \cdot \beta\right] = B_i b_i, \tag{11}$$

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

So the Bayes estimate $\psi^* = (\beta_1^*, \ldots, \beta_I)^T$ is given by $\psi^* = Bb$

with $B^{-1} = Z_2^T (V + \Omega)^{-1} Z_2 + \Lambda^{-1} = diag\left\{ B_1^{-1}, \ldots, B_I^{-1} \right\}$ and

$b = Z_2^T (V + \Omega)^{-1} \zeta + \Lambda^{-1} Z_3 \beta = (b_1, \ldots, b_I)^T$.

Hence, we obtain the Bayes estimates β_i^* as given in (11), with means

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

and covariance matrices

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

The posterior distribution of the parameter vectors $\theta_i = (\beta_{i1}, \ldots, \beta_{iK})^T$, $i = 1, \ldots, I$, given ζ, β, Ω and Λ are p-variate normal, $p = 4$, with means ϕ_i^* and covariance matrices D_i. Thus the Bayes estimate θ^* is given by

$\theta^* = Dd$ or rather

$$\theta_i^* = \left(\beta_{i1}^*, \ldots, \beta_{iK}^* \right)^T = D_i d_i \ , \quad i = 1, \ldots, I \tag{12}$$

with $D^{-1} = V^{-1} + \left\{ \Omega + Z_2 \Lambda Z_2^T \right\}^{-1} = diag\left\{ D_1^{-1}, \ldots, D_I^{-1} \right\}$ and

$d = V^{-1} \zeta + \left\{ \Omega + Z_2 \Lambda Z_2^T \right\}^{-1} Z_2 Z_3 \beta = (d_1, \ldots, d_I)^T$, where

$$D_i^{-1} = \begin{pmatrix} \left(\tau_{i1}^2 C_{i1} \right)^{-1} & & 0 \\ & \ddots & \\ 0 & & \left(\tau_{iK}^2 C_{iK} \right)^{-1} \end{pmatrix} + \left\{ \begin{pmatrix} \Omega_i & & 0 \\ & \ddots & \\ 0 & & \Omega_i \end{pmatrix} + \begin{pmatrix} \Sigma & \cdots & \Sigma \\ \vdots & \ddots & \vdots \\ \Sigma & \cdots & \Sigma \end{pmatrix} \right\}^{-1} \quad \text{and}$$

$$d_i = \begin{pmatrix} \left(\tau_{i1}^2 C_{i1} \right)^{-1} & & 0 \\ & \ddots & \\ 0 & & \left(\tau_{iK}^2 C_{iK} \right)^{-1} \end{pmatrix} \begin{pmatrix} \zeta_{i1} \\ \vdots \\ \zeta_{iK} \end{pmatrix} + \left\{ \begin{pmatrix} \Omega_i & & 0 \\ & \ddots & \\ 0 & & \Omega_i \end{pmatrix} + \begin{pmatrix} \Sigma & \cdots & \Sigma \\ \vdots & \ddots & \vdots \\ \Sigma & \cdots & \Sigma \end{pmatrix} \right\}^{-1} \begin{pmatrix} \beta \\ \vdots \\ \beta \end{pmatrix} .$$

The estimators are normally distributed with means

$$E\left(\theta_i^*\right) = D_i \cdot \begin{pmatrix} \left(\tau_{i1}^2 C_{i1}\right)^{-1} & & 0 \\ & \ddots & \\ 0 & & \left(\tau_{iK}^2 C_{iK}\right)^{-1} \end{pmatrix} \begin{pmatrix} \beta_{i1} \\ \vdots \\ \beta_{iK} \end{pmatrix} + \left\{ \begin{pmatrix} \Omega_i & & 0 \\ & \ddots & \\ 0 & & \Omega_i \end{pmatrix} + \begin{pmatrix} \Sigma & \cdots & \Sigma \\ \vdots & \ddots & \vdots \\ \Sigma & \cdots & \Sigma \end{pmatrix} \right\}^{-1} \begin{pmatrix} \beta \\ \vdots \\ \beta \end{pmatrix}$$

and covariance matrices

$$Cov\left(\theta_i^*\right) = D_i \cdot diag\left\{ \left(\tau_{i1}^2 C_{i1}\right)^{-1}, \ldots, \left(\tau_{iK}^2 C_{iK}\right)^{-1} \right\} \cdot D_i^T \ .$$

As β will be unknown in the practical application we replace it in (11) and (12) by its Bayes estimate β^*.

The previous estimators are based on known covariance matrices. 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 need a method to estimate both the parameter vectors and the covariance matrices. Such a method is presented in the following section.

Estimators in the case of unknown covariance matrices

In the case of unknown variances τ_{ik}^2, $i = 1, \ldots, I$, $k = 1, \ldots, K$, 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 $p\left(\tau_{ik}^2\right) \propto 1$, 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

$$\hat{\tau}_{ik}^2 = \frac{1}{J} \cdot \sum_{j=1}^{J} \left(y_{ijk} - f\left(\zeta_{ik}, t_j\right)\right)^2 \qquad , i = 1, \ldots, I,\ k = 1, \ldots, K. \tag{13}$$

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 Ω and Λ. We adapt this algorithm to our four stage model 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. 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 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

$$\begin{aligned}
&\left(\prod_{i=1}^{I}\prod_{k=1}^{K}\left|\hat{\tau}_{ik}^2 C_{ik}\right|^{-1/2}\right)\cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}\sum_{k=1}^{K}\frac{1}{\hat{\tau}_{ik}^2}\left(\zeta_{ik}-\beta_{ik}\right)^T\cdot C_{ik}^{-1}\left(\zeta_{ik}-\beta_{ik}\right)\right\}\cdot\\
&\left(\prod_{i=1}^{I}\left|\Omega_i\right|^{-K/2}\right)\cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}\sum_{k=1}^{K}\left(\beta_{ik}-\beta_i\right)^T\Omega_i^{-1}\left(\beta_{ik}-\beta_i\right)\right\}\cdot\\
&\left|\Sigma\right|^{-I/2}\cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}\left(\beta_i-\beta\right)^T\Sigma^{-1}\left(\beta_i-\beta\right)\right\}\cdot\\
&\prod_{i=1}^{I}\left|\Omega_i\right|^{-1/2(\rho_1-p-1)}\cdot \exp\left\{-\frac{1}{2}\sum_{i=1}^{I}tr\left(R_1^{-1}\cdot\Omega_i^{-1}\right)\right\}\cdot\\
&\left|\Sigma\right|^{-1/2(\rho_2-p-1)}\cdot \exp\left\{-\frac{1}{2}tr\left(R_2^{-1}\cdot\Sigma^{-1}\right)\right\}
\end{aligned}\tag{14}$$

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, $\beta^{(l)}$, $\psi^{(l)} = \left(\beta_1^{(l)}, \ldots, \beta_I^{(l)}\right)^T, \theta^{(l)} = \left(\beta_{1,1}^{(l)}, \ldots, \beta_{IK}^{(l)}\right)^T$, $\Omega^{(l)}$ and $\Lambda^{(l)}$, by replacing the covariance matrices in (3.1), (3.2), and (3.4) 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 modes using $\beta^{(l)}$, $\psi^{(l)}$, and $\theta^{(l)}$ (M-Step).

<u>E-Step</u>

Approximating Ω and Λ in (10) by $\Omega^{(l-1)}$ and $\Lambda^{(l-1)}$ we obtain

$$\beta^{(l)} = \left[Z_3^T Z_2^T \left\{\hat{V} + \Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} Z_2 Z_3 \right]^{-1} Z_3^T Z_2^T \left\{\hat{V} + \Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} \zeta \ , \tag{15}$$

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 in (3.2) by $\beta^{(l)}$, $\Omega^{(l-1)}$, and $\Lambda^{(l-1)}$, respectively, yields

$$\psi^{(l)} = \left[Z_2^T \left(\hat{V} + \Omega^{(l-1)}\right)^{-1} Z_2 + \Lambda^{(l-1)^{-1}} \right]^{-1} \left[Z_2^T \left(\hat{V} + \Omega^{(l-1)}\right)^{-1} \zeta + \Lambda^{(l-1)^{-1}} Z_3 \beta^{(l-1)} \right]. \tag{16}$$

In the same way we get $\theta^{(l)}$ by replacing the unknown parameters by their current estimates in (12):

$$\theta^{(l)} = \left[\hat{V}^{-1} + \left\{\Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1}\right]^{-1} \left[\hat{V}^{-1} \zeta + \left\{\Omega^{(l-1)} + Z_2 \Lambda^{(l-1)} Z_2^T \right\}^{-1} Z_2 Z_3 \beta^{(l)} \right]. \tag{17}$$

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 (14) is given by

$$\Omega_i^{(l)} = \frac{R_1^{-1} + \sum_{k=1}^{K}\left(\beta_{ik}^{(l)} - \beta_i^{(l)}\right)\cdot\left(\beta_{ik}^{(l)} - \beta_i^{(l)}\right)^T}{K + \rho_1 - p - 1}, \qquad i = 1, \ldots, I, \text{ and} \tag{18}$$

$$\Sigma^{(l)} = \frac{R_2^{-1} + \sum_{i=1}^{I}\left(\beta_i^{(l)} - \beta^{(l)}\right)\left(\beta_i^{(l)} - \beta^{(l)}\right)^T}{I + \rho_2 - p - 1} \tag{19}$$

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

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

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

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 Hierarchical model for group B

Analysing the experiments of group B it has to be taken into account that the initial concentration varies from occasion to occasion. Thus the individual and day-dependent initial concentration $y_{ik}(0)$ varies across a day-dependent mean $y_k(0)$, about 20 ppm for $k = 1$, for instance. Therefore the model for group A has to be modified for the experimental design of group B.

Nonlinear hierarchical model

As we are merely interested in the kinetic parameter we ignore the potential dependence between their estimates and the initial concentration. Otherwise we would receive a more complex model which would be much more difficult to estimate as it was the case for model A. Moreover, assuming overall first order kinetics implies this independence, although we have to verify this assumption, of course. A suitable test will be presented in a further paper. Thus, we developed our hierarchical model only for the kinetic parameters using the Maximum-Likelihood estimates of the initial concentration if necessary, i.e. for the calculation of the residuals.

Hence, 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 $\varphi_{ik}, y_{ik}(0), \tau^2_{ik}$: $y_{ijk} \sim N(f(\varphi_{ik}, y_{ik}(0), t_j), \tau^2_{ik})$ $i = 1, \ldots, I, j = 1, \ldots, J$ and

$k = 1, \ldots, K$, with $\beta_{ik} = (\varphi^T_{ik}, y_{ik}(0))^T$, and $\varphi_{ik} = (k_{12ik}, k_{21ik}, k_{elik})^T$

given φ_i, Ω_i: $\varphi_{ik} \sim N(\varphi_i, \Omega_i)$, $i = 1, \ldots, I$ and $k = 1, \ldots, K$,

with $\varphi_i = (k_{12i}, k_{21i}, k_{eli})^T$,

given φ, Σ: $\varphi_i \sim N(\varphi, \Sigma)$ $i = 1, \ldots, I$,

with $\varphi = (k_{12}, k_{21}, k_{el})^T$

$p(\varphi) \propto 1$ $\forall\ \varphi \in \mathbb{R}^3$.

Linear hierarchical model

The nonlinear hierarchical model is transformed into a linear one by substituting the observations y_{ijk} by the Maximum-Likelihood estimates ζ_{ik}. Thus, we receive the following linear model:

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

where $\tilde{\zeta} = (\tilde{\zeta}_{1,1}, \ldots, \tilde{\zeta}_{IK})^T$, $\tilde{\zeta}_{ik} = \left(\hat{k}_{12ik}, \hat{k}_{21ik}, \hat{k}_{elik}\right)^T$ are the three first components of the Maximum-Likelihood estimate ζ_{ik} of β_{ik}, $\theta = (\theta_1, \ldots, \theta_I)^T$, $\theta_i = (\varphi_{i,1}, \ldots, \varphi_{iK})^T$, $V = diag\{(\tau_{1,1}^2 \tilde{C}_{1,1}), \ldots, (\tau_{IK}^2 \tilde{C}_{IK})\}$, and $\tau_{ik}^2 \tilde{C}_{ik}$ denotes the left upper 3×3 matrix of the inverse of the Information matrix $\left(\tau_{ik}^2 C_{ik}\right)^{-1}$.

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

where , $\theta = (\theta_1, \ldots, \theta_I)^T$, $\theta_i = (\varphi_{i,1}, \ldots, \varphi_{iK})^T$, $\psi = (\varphi_1, \ldots, \varphi_I)^T$

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

given φ, Λ: $\psi \sim N(Z_3\varphi, \Lambda)$,

where $\varphi = (k_{12}, k_{21}, k_{el})^T$, $\Lambda = diag\{\Sigma, \ldots, \Sigma\}$, and $Z_3 = (I_3, \ldots, I_3)^T$ is a suitable design matrix,

$p(\varphi) \propto 1$, $\forall \varphi \in \mathbb{R}^3$.

The Bayes estimates θ^*, ψ^*, and φ^* are the same as the estimates in section 3.2 for group A. Note, that using (10) to (12) in the case of known covariance matrices or rather (15) to (19) in the case of unknown covariance matrices that the dimension p of the parameter vectors is three instead of four.

5. 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 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.

A further step in the reduction of complexity was the presentation of a simpler model for a repeated measurement design which ignored the correlation between β_{i1}, β_{i2}, . . ., and β_{iK} (Selinski and Urfer,1998; Selinski et al., 1999). Assuming independence between β_{i1}, β_{i2}, . . ., and β_{iK} estimates for each parameter vector β_{ik} can be calculated separately and the estimation of β_{ik} and β requires only the inversion of matrices of size 4×4. Moreover, it has to be checked if the estimation procedure for model A can be improved by considering only the kinetic parameters like in model B. The size of the matrices which have to be inverted numerically would reduce to 15×15.

Including the initial concentration as parameter in the hierarchical model of group B leads to a more complicated model which requires the calculation of inverse matrices of size 35×35.

As with increasing size of matrices the error increases exponentially the question is which model has to be preferred: a quite simple model ignoring the dependency between the parameter vectors, a more complex model which ignores the initial concentration as parameter or a model requiring matrix manipulations which produce possibly huge errors? Furthermore, it has to be checked which model copes better with outliers, missing data and departure from first order kinetics.

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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