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Physiologically based pharmacokinetic modeling in risk assessment

Development of Bayesian population methods

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List of publications

This thesis is based on the publications listed below, referred to in the text by their Roman numerals. The papers are reprinted with the kind permission of the publishers of the journals.

I. Jonsson F, Bois F Y, Johanson G. Assessing the reliability of PBPK models using data from methyl chloride-exposed, non-conjugating human subjects. *Arch Toxicol., in press*. (doi 10.0007/s002040100221)

II. Jonsson F, Bois F Y, Johanson G. Physiologically based pharmacokinetic modeling of inhalation exposure of humans to dichloromethane during moderate to heavy exercise. *Toxicol. Sci.,* (2001), 59:209-218.

III. Jonsson F, Johanson G. Bayesian estimation of variability in adipose tissue blood flow in man by physiologically based pharmacokinetic modeling of inhalation exposure to toluene. *Toxicology,* (2001), 157:177-193.

IV. Jonsson F, Johanson G. A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. *Submitted*.

V. Jonsson F, Johanson G. Physiologically based modeling of the inhalation kinetics of styrene in humans using a Bayesian population approach. *Submitted*.

Abbreviations

ATBF	Adipose tissue blood flow (ml/min/100 g fat)
BHt	Body height (cm)
blo	Subscript denoting venous blood
BV	Lean body volume (l)
BWt	Body weight (kg)
Cf	Coefficient for scaling to physiological quantity
CV	Coefficient of variation
DCM	Dichloromethane
exh	Subscript denoting exhaled air
FFM	Fat free mass (kg)
h	Subscript denoting hepatic
km	Michaelis constant (µmol/l)
m	Subscript denoting muscle
μ	Vector of "true" mean population parameters
MCMC	Markov chain Monte Carlo
PBPK	Physiologically based pharmacokinetic
PC	Partition coefficient
pfat	Subscript denoting perirenal fat
pul	Subscript denoting pulmonary
Q	Flow (l/min)
scfat	Subscript denoting subcutaneous fat
SD	Standard deviation
Σ	"True" population variances of parameters in the population
TBW	Total body water (l)
θ	Vector of unknown individual parameters
tot	Subscript denoting total
V	Compartment volume (l)
Vmax	Maximum rate of metabolism (µmol/min)
wm	Subscript denoting working muscle
wp	Subscript denoting well-perfused tissue
ΔV_{O2}	Excess Oxygen uptake above rest (l/min)
ε	Residual error encompassing intra-individual variability and measurement error

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1. Introduction

It is virtually impossible to completely ban hazardous chemicals from the occupational environment. Costs, monetary or otherwise, are associated with every regulation or substitution activity. These costs must be balanced against the benefits of a reduced health hazard. An evaluation of these cost/benefit ratios involves a numerical assessment of health hazards, *i.e.* risk assessment.

In risk assessment of chemicals, the most commonly used dose measures are the external exposure levels. However, the health hazard of a pollutant is more closely related to the internal exposure delivered at a critical target in the body than to the external exposure. For some chemicals, toxicity may be associated with metabolic activation to a more reactive species. The metabolic activation may be subject to saturation at high doses, and thus result in a nonlinear relationship between external exposure and toxic risk. In the field of risk assessment, the internal exposure at the target site for toxic effect is often referred to as a "target dose". The most convenient way of calculating the target dose is by the use of a toxicokinetic (or pharmacokinetic) model (46). Toxicokinetic models summarize the behavior of chemicals in the body, *e. g.*, the processes of absorption, distribution, metabolism and elimination. Factors that are known or expected to have an influence on the target dose, including enzyme inhibition, physical workload, exposure route, etc, may be accounted for in the model. Furthermore, variability in target dose may be estimated by introducing variability in model parameters, thereby assessing the target dose distribution in a simulated population.

1.1 Structural Models

A variety of kinetic models have been suggested to describe disposition of a chemical within a body. In these models, the disposition in the human body is given a simplified description as movement of chemical between compartments. There are two main classes of compartmental models in the literature, namely empirical (classical) models, and physiologically based models.

1.1.1 Empirical models

In classical pharmacokinetic models, the body is represented by several, but relatively few, connected compartments. Each compartment is designed as a space, without any explicit physiological meaning, where the chemical is assumed to be distributed homogeneously. The transfer of chemical between compartments is described by a system of difference or differential equations. A number of parameters such as clearance and volume of distribution describe transfer of chemical between compartments. These parameters generally lack any explicit physiological meaning, but may be explained in terms of binding to various tissues, plasma proteins or distribution in the interstitial tissue water. The number

of compartments and the values of the parameters governing the rate of exchange are determined by the fitting of the model to the kinetic data. A typical approach when choosing among structural models is to start with a simple one-compartment model, and then add compartments as long as the goodness-of-fit plots show bias, which is commonly interpreted as a sign of structural misspecification. These models are often referred to as empirical or data-based models. As an example, a typical two-compartment model is depicted in Figure 1.

Empirical models are useful tools for drawing conclusions from the current data, and are widely used in pharmacokinetic studies to investigate drug disposition in the body. In addition, the disposition of a pharmaceutical drug in the body tends to be less complicated than the disposition of a hazardous chemical, as the distribution profile of the drug is usually monitored quite closely, and is a matter of concern, during drug development. As empirical models are highly dependent on the data used for calibration and often lack direct physiological meaning, these models are not suitable for the extrapolation of kinetic results between species or from *in vitro* to *in vivo* conditions. In risk assessment, such extrapolations are often needed, as toxicokinetic data from humans are lacking in most cases, due to time, cost and most importantly, the perceived risks associated with experimental exposure of humans. For these applications, physiologically based models have been developed.

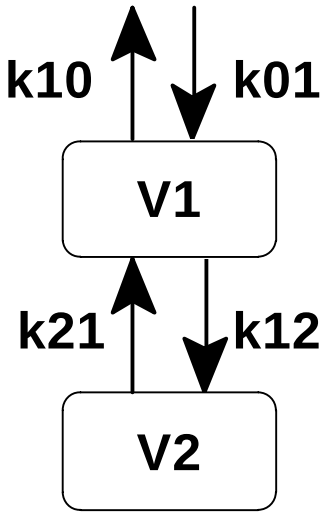

Figure 1. Example of an empirical 2-compartment pharmacokinetic model.

1.1.2 Physiologically based pharmacokinetic models

In physiologically based pharmacokinetic (PBPK) models, the body is subdivided into a series of anatomical or physiological compartments that represent specific organs or lumped tissue and organ groups. The transfer of chemical between compartments is described by a set of differential equations. The parameters of the model are of three types: Physiological parameters such as tissue perfusions or

tissue volumes, physicochemical parameters such as partition coefficients that describe the degree of partitioning of a given chemical to a given tissue, and biochemical parameters describing metabolic processes. An example of a PBPK model is given in Figure 2.

In most PBPK models, distribution in a given compartment is assumed to be limited by perfusion. Once in a compartment, the chemical is assumed to distribute evenly and homogeneously throughout the compartment volume. However, several more complex models, where diffusion-limited compartment distribution is assumed in some (23, 53) or all (41) compartments, have also been suggested, primarily for rodents. The standard assumption of flow-limitation has been put into question (54).

The structure of a PBPK model is determined by the intended use of the model, the biochemical properties of the chemical studied and the effect site of concern.

Much attention has been given to PBPK models in pharmaceutical research (65-67), as such models facilitate *in vitro-in vivo* extrapolation (3, 74) in early stages of drug development.

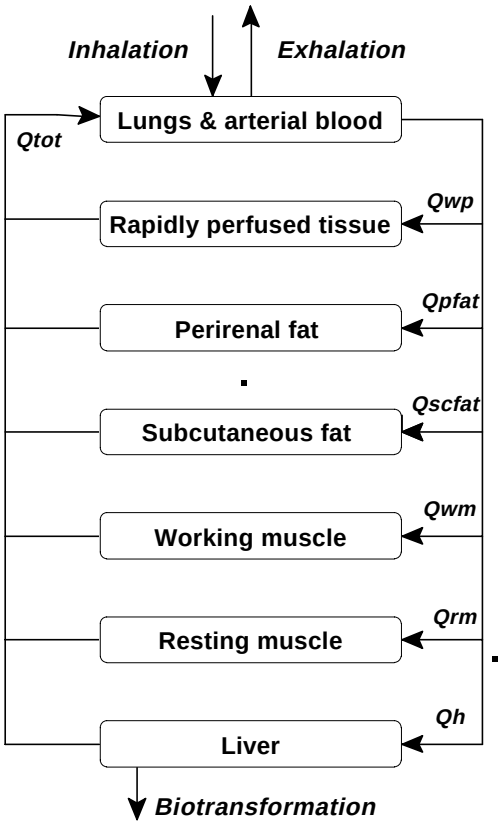

Figure 2. Example of a PBPK model. This structural model was used in studies III, IV and V, where concentrations in subcutaneous fat were included in the analyses.

When chemicals of risk are modeled, compartments for fat tissue, liver, and poorly and richly perfused tissue are usually included. However, in theory there is no limit as to how complicated PBPK models can be, and there are examples in

the literature of models containing more than 20 compartments, including those describing the disposition of several metabolites.

In a workplace, the most important exposure route is via inhalation. Inhalation exposure to volatile chemicals is complicated and needs special attention. In PBPK models for inhalatory uptake, the exchange of solvent between blood and alveolar air is usually assumed to be very rapid and it is also assumed that all exchange occurs in the alveoli and not in other parts of the respiratory tree. The first assumption is plausible, as volatiles are small, non-charged molecules, which easily penetrate the cell membranes. The latter assumption has been questioned for volatiles in general with respect to rodents (47) and for polar volatiles with respect to prealveloar deposition during inhalation and release during exhalation (a wash-in wash-out effect) and prealveolar uptake (45).

PBPK models are frequently used in simulation studies using animal- and/or *in vitro*-derived parameter values, without any calibration at all. However, since these models are simplifications of complicated biological processes, there is some uncertainty associated with their predictions. Typically, adjustment of model parameters by the means of some sort of calibration process is needed to describe experimental toxicokinetic data accurately. However, while PBPK models have now become firmly established tools for chemical risk assessment (38, 56), the development of a strong statistical foundation to support PBPK model calibration and use has received relatively little attention. Until recently, there was no method available for rigorous statistical validation of PBPK models. A very common calibration method is the adjustment of one or two model parameters, while assuming population mean values on all other model parameters (58). This is understandable, given that experimental data alone are usually insufficient to estimate all PBPK parameters simultaneously using the standard maximum likelihood techniques usually employed in pharmacokinetic modeling. These techniques are implied by software packages such as ASCL (AEgis Technologies Group, Huntsville, AL), WinNonlin (Pharsight Co., Mountainview, CA) or Nonmem (5). The parameterization is often empirical, and no statistically sound estimates of the uncertainty of parameters or model predictions are derived. In addition, when only a select few (usually the metabolic) parameters are estimated, the derived estimates are conditional on the assumed values for all fixed parameters. Yet the exact values of the physiological and physicochemical parameters in humans are not known with precision, especially not *in vivo*. The uncertainty tends to be inflated in the metabolic parameters, while being ignored in others (91). In order to properly account for the inter- and intra-individual variabilities inherent in the toxicokinetic data, and entangle these variabilities (a biological reality) from uncertainty (lack of data), it is advisable to use a statistical model. The statistical model may describe the relationships between the individual and population parameters, and makes it possible to estimate the population variability (76).

1.2. Hierarchical population models

Whenever one wants to make inference about the kinetic behavior of a certain chemical in the general population, it is necessary to derive quantitative information from kinetic data collected at the individual level.

There are several approaches to this problem. The simplest is often referred to as naive pooling, and amounts to pooling of data from many subjects and subsequent model calibration against mean concentrations in the sample population over time. By the use of this method, only an estimate of the theoretical mean behavior of the chemical in the general population is derived, and a rather shaky one, as a number of variance components are completely ignored. A better method is the two-stage method, where the model is fit to the data from different individuals separately. The population kinetics are then summarized by performing descriptive statistics on the individual parameter estimates. When the two-stage approach is used, reasonable estimates of population kinetics may be derived. However, there are a number of limitations to this method. The combination of estimates is made without any statistical model for the inter-individual variability. No use is made of the information present in the kinetic profiles of subjects other than the one being estimated at the time, and thus, the data are not used to their fullest potential with regards to information content (77).

While more sophisticated two-stage models have been suggested, what is generally regarded as the most convenient approach is to use a hierarchical structure to distinguish intra-individual variability from variability at the population level. Population analyses are firmly established tools in the context of evaluation and development of pharmaceutical drugs (92). The basic idea of a population model is that the same compartmental model can describe the concentration-time profiles in all individuals, and that the model parameters can vary from individual to individual. The individual parameter sets are assumed to have arisen from a theoretical population distribution. The population distribution may be assumed to be known or unknown. The former approach, which is the most common, is referred to as parametric, and the population parameters are estimated in the analysis conditional on the assumed shape of the distribution. In non-parametric methods, the population distribution, both with regards to the parameter values and the shape of the distribution, is investigated. Oftentimes, the population sample is too small for any meaningful application of a non-parametric approach.

The advantages of the population approach have long been discussed in the context of pharmaceutical drugs (81). It has also been shown that the population approach is preferable to other methods, such as the two-stage approach, even when the population sample is very small (49). Despite these firmly established advantages of a hierarchical approach in pharmacokinetic modeling, there has been very little attention to this approach in the toxicology literature, with the exception of the work presented by Bois and co-workers using a Bayesian approach (10-13, 15). Droz and co-workers developed a population PBPK model for risk assessment (29, 30), but did not make any calibration of the model to

toxicokinetic data. There are by now also some other recent examples in the literature of population modeling of toxicokinetic data *e.g.* (50, 73), which indicates that these approaches are gaining momentum in the toxicology community. However, there is still a need for widespread use of methods that address the uncertainties inherent in toxicokinetic data and the variability in the human populations for which risk predictions are made and incorporates these issues into the fitting of PBPK models to toxicokinetic data.

1.3. Statistical approaches

Whenever conclusions are made based on collected data, it is necessary to resort to some sort of statistical foundation in order to assure that the conclusions are reasonable. By far the most common statistical approach to analyze scientific research is what is known as the frequentist or "classical" method. However, there exists another option, namely the Bayesian approach.

1.3.1. The frequentist approach

In a frequentist analysis, the probability (p) of observing results (or data, y) as or more extreme than the one in the present study, given a certain assumption (θ), is assessed. An example is in the analysis of clinical trials, where focus is on assessment of the probability of collecting data as or more extreme as those in the present study, given that the null hypothesis is true. This probability is often summarized as a p-value and may be written as

$$p(y|\theta)$$

This function is called the *likelihood function*. The assumption θ may be one of a certain difference between study groups, or a certain value of a parameter. In the case of modeling, the parameter values for which the data are most likely to have arisen are derived by the use of a maximum likelihood estimator. The maximum likelihood estimator reports the parameter values as point estimates with associated standard deviations or confidence intervals.

A frequentist analysis tends to ignore the results in previous studies, or for that matter any external evidence other than the data collected in the present study, and may thus very well produce totally unrealistic results, in view of the previous knowledge. Likewise, the measures of uncertainty produced in a frequentist analysis only takes the data collected in the present study into account. The plausibility of the results from a frequentist analysis is often a cause for concern, and the results of the analysis must always be assessed with regards to the previous research by the researcher post-analysis.

In the case of PBPK modeling, the available toxicokinetic data are usually relatively sparse, considering the elaborate model structure, with many parameters that are impossible to estimate independently when only the present toxicokinetic data are taken into account. However, some knowledge about many parameters of

the model may be gathered from the available reference literature. This knowledge is associated with different degrees of uncertainty. The mean value of the unit perfusion of liver tissue may be known with a fair amount of precision, and there may also be a reasonable estimate of the level of inter-individual variability of that perfusion available. On the other hand, there may be very little data on the mean metabolic capacity for a given chemical in a human population. There may only be an educated guess, based on animal-to-human extrapolation, available. These different levels of uncertainty are difficult to account for properly using the frequentist approach, as the frequentist analysis only performs an assessment of the likelihood function, and ignores the external evidence.

1.3.2. The Bayesian approach

In a Bayesian analysis, the focus is on the probability of the assumptions, given the available data, rather than the other way around. In order to assess the probability of the assumptions, we must incorporate the previous knowledge into the analysis by defining the probability of our assumptions ($p(\theta)$), before taking the data from the present study into account. This probability is referred to as the *prior probability.* The basic tool of a Bayesian analysis is Bayes' theorem, which tells us how to update our belief on a certain assumption based on our observations. The *posterior probability* of the assumption θ given data y is given by:

$$p(\theta|y) = \frac{p(\theta) \bullet p(y|\theta)}{p(y)}$$

The function $p(y \mid \theta)$ is the likelihood function already discussed in the previous section. Bayes' theorem expresses our uncertainty of θ after taking the data, as well as external evidence, into account. The theorem also tells us how to calculate the posterior distribution:

$$p(\theta|y) = \frac{p(\theta) \bullet p(y|\theta)}{\int p(\theta) \bullet p(y|\theta) d\theta}$$

Oftentimes, it is not necessary to calculate the denominator, and the theorem may be rewritten as

$$p(\theta|y) \propto p(\theta) \bullet p(y|\theta)$$

A Bayesian approach makes it possible to merge *a priori* knowledge from the literature with the information in experimental toxicokinetic data. As everything in a Bayesian analysis is based on probabilities, such an analysis yields estimates in the shape of statistical distributions (called "posterior densities"), of the parameter values, rather than single point estimates with a standard deviation. These posterior estimates are consistent with both the experimental data and the prior

knowledge (specified as “prior distributions” of the parameters), as postulated in the theorem.

If combined with hierarchical modeling, the Bayesian approach yields posterior estimates of the parameters for each subject, as well as for the population parameters (87).

As the Bayesian approach is prediction and decision oriented, it is particularly suitable in the area of PBPK modeling in risk assessment. However, the Bayesian approach is surrounded by some controversy. One common objection is that the Bayesian approach is subjective. Different researchers may make different assessments of previous results, and may thus assign different priors. However, considerable subjectivity also goes into frequentist analyses in terms of model choice, statistical tests, confidence levels, etc. In addition, differences in opinion are not very rare in the field of scientific research. It may be argued that an approach that openly acknowledges the researcher’s subjectivity is more honest than the frequentist approach, which tends to give a false sense of objectivity (8).

1.4. Markov chain Monte Carlo simulation

In the context of a Bayesian analysis of population PBPK models, we are interested in sampling from the posterior target distribution $p(\theta|y)$ in order to make inference to the general population. As the models are quite complex, independent sampling is very difficult. The solution to this problem is to perform dependent random (Monte Carlo) sampling from the prior distributions and use these samples as starting points for Markov chains. As the iterations progress, the Markov chain converges to the posterior target distribution $p(\theta|y)$. This is Markov chain Monte Carlo (MCMC).

There are various MCMC techniques available (40). The Metropolis-Hastings algorithm has previously been shown to be effective when dealing with PBPK models (13, 14). In MCMC, all model unknowns are assigned starting values by random sampling from prior distributions, as mentioned above. When the Metropolis-Hastings algorithm is used, each component θ_k of the parameter vector θ is updated at each iteration step according to an adaption/rejection rule. A candidate point θ_k^* is sampled from a jumping distribution at iteration *t*. The ratio of densities,

$$r = \frac{p(\theta^{*}|y)}{p(\theta^{t-1}|y)}$$

is then calculated, and if the ratio *r* exceeds 1, the new value θ_k^* is accepted and replaces θ_k, otherwise the old value is kept. After sequential updating of all the components of θ, their current values are recorded, and an iteration of the Markov chain is completed. In a typical case, many (several thousands) of iterations are needed. An example of the MCMC process is given in Figure 3.

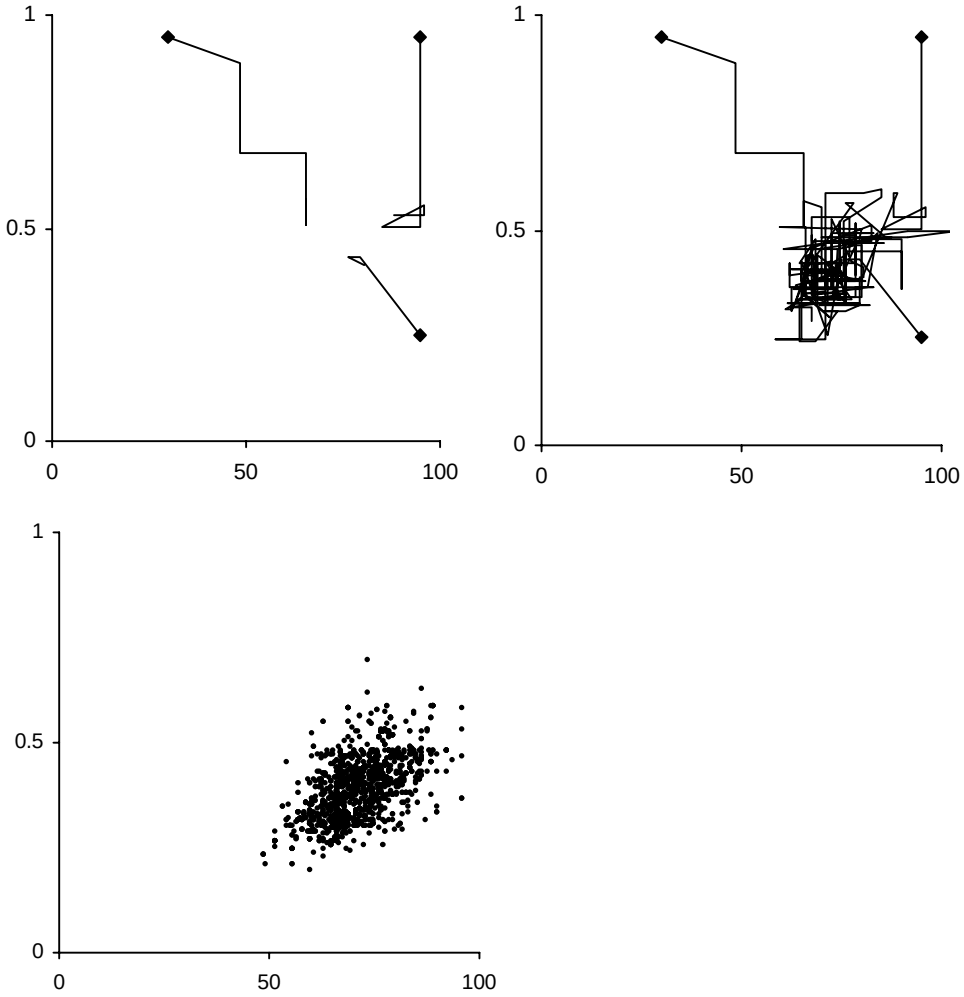

Figure 3. Example of Markov chain Monte Carlo simulation. The simultaneous trajectories for two parameters in three independent Markov Chains performing a random walk through the joint distribution of the parameters are shown. (a) After 50 iterations, the chains are still far from convergence with regards to these two parameters. (b) After 500 iterations, convergence is approaching. (c) After 1,000 iterations, the three chains are at convergence. Only the last 500 iterations of each chain are shown, and the pooled joint posterior distribution is shown, rather than the trajectories of the chains.

When several independent, overdisperse chains are run, they converge to the target distribution. Several criteria have been suggested for convergence assessment. Gelman and Rubin (39) introduced the symbol $\hat{R}$ to describe the estimated (^) scale Reduction (*R*). At perfect convergence, all $\hat{R}$ values should be equal to 1. An $\hat{R}$ of 1.3, for example, indicates that the use of longer chains would have reduced the parameter uncertainty, quantified as variance estimates, by 30 per cent.

When convergence is achieved, the samples generated by running the chains further may be considered approximate samples from the joint target distribution, and may thus be used to make inference.

While MCMC is a Monte Carlo-based technique, it should not be confused with the regular Monte Carlo techniques commonly employed for predictions, for example in conjunction with PBPK models (85). Regular or simple Monte Carlo techniques are used to estimate variability in model output by sampling from the prior distribution in order to make an assessment of the sensitivity of model output

(such as target dose) to these prior assumptions. However, no updating of the prior belief is performed in such Monte Carlo simulations. The MCMC technique may be viewed as an extension of this practice. It should also be noted that if uniform, *i.e.* non-informative priors (complete ignorance about plausible values) are used, the posterior will be proportional to the likelihood of the data, and in the end equivalent to the standard likelihood-based (frequentist) approach to PBPK modeling. It should also be noted that if the data do not convey any information at all on the parameters, the posterior distributions will be equivalent to the prior.

1.5. Available toxicokinetic data

When occupational exposure to volatiles occurs in the workplace, it is usually in conjunction with some sort of physical labor. During physical exercise, alveolar ventilation increases, as well as the perfusion of several tissue groups. This has a significant effect on the uptake of volatile. However, most human experimental inhalation exposures to volatiles are performed at rest. It is currently not standard practice to account for the effect of physical workload when PBPK models are used to estimate target dose, although in occupational exposure.

Since the early seventies, a large number of experimental inhalation studies of the kinetics of several volatiles in human volunteers have been performed at the National Institute for Working Life in Solna. Extensive data on simultaneous concentrations of volatile in venous and arterial blood, urine, fat tissue and end-exhaled air were collected in conjunction with these experiments, along with information on various covariates, such as oxygen uptake over time and body weight. Generally, the exposures were conducted at some level of physical workload, and subjects were exposed via inhalation.

These data are unique, even in an international perspective, as the exposures were conducted during various levels of physical workload and at high exposure levels, and with simultaneous monitoring in several tissues and body fluids both during and post-exposure.

To this day, only very limited analyses of these data have been performed.

1.6. Previous Bayesian population PBPK modeling

There has been very little attention to the Bayesian approach in the scientific literature on PBPK modeling in risk assessment. The only quantitative work on Bayesian calibration of PBPK models is that performed by Bois and co-workers *e.g.* (10-13). That work also represents the only studies on the calibration of population models in risk assessment. Dr. Bois is also the co-author of MCSim (14), a software for MCMC simulation of nonlinear models, and also of a recent article discussing the advantages of Bayesian PBPK modeling comprehensively (7).

In order to evaluate the Bayesian population approach to population PBPK modeling more extensively, it is of interest that more studies along these lines are undertaken. In addition, since the standard, frequentist, approach to PBPK

modeling has such poor ability to quantify or distinguish uncertainty from inter- or intra-individual biological variability, any hierarchical PBPK modeling using the Bayesian approach may be considered an important addition to the published literature on PBPK modeling.

The population model suggested by Droz and co-workers (29, 30) contained a number of interesting features with respect to equations describing the intra-individual variability in tissue perfusion with physical exercise. These equations have never been validated in a rigorous statistical analysis. Furthermore, the work of Bois and co-workers, although very impressive, only encompasses a limited amount of data on a limited amount of solvents.

2. Aims

The main purpose of this thesis was to apply Bayesian population techniques for PBPK modeling to toxicokinetic data from studies performed previously at the National Institute for Working Life. Data from human exposures to methyl chloride, dichloromethane, toluene, and styrene are included. These chemicals were chosen among those for which toxicokinetic data were available at the Institute with regards to the perceived health risks, the availability of previous PBPK modeling studies, and their estimated information content in these data sets with regards to the estimation of physiological parameters. In addition to the derivation of significant information on the population kinetics of these particular volatiles, objectives of the thesis work include:

- Validation of the available equations describing intra-individual variability of tissue perfusion in conjunction with physical exercise, and assessment of the inter-individual variability in these changes.

- The ability of PBPK models to predict concentrations in sampled adipose tissue.

- Incorporation of models for risk assessment into the Bayesian PBPK framework.

- General conclusions with regards to the calibration of PBPK models and their application in risk assessment.

3. Methods

3.1. Experimental data

Generally, the exposures were conducted at some level of physical workload, as subjects were exposed via inhalation, and it is difficult to standardize inhalation uptake at rest due to intra-individual variability in alveolar ventilation at rest. Note also that in the present thesis, all collected samples of exhaled air are denoted "end-exhaled air", as the collected air represent the concentrations of volatile in the last fraction of an exhaled breath, and may or may not be truly "alveolar".

3.1.1. Methyl chloride (Study I)

Methyl chloride is a gas that was formerly used as a coolant, and to some extent as a local anaesthetic. It is now primarily used in production processes, and as an intermediate solvent in the production of plastics, pharmaceuticals, herbicides, pigments and disinfectants (60). Present occupational exposure level limit value in Sweden is 10 ppm (short-term exposure 20 ppm).

Eight subjects, five male and three female, were exposed to methyl chloride (10 ppm, 120 min) in an exposure chamber during light physical exercise (63). Frequent blood sampling was performed during and up to 4 hours post-exposure. Arterialised capillary blood was collected from the pre-warmed fingertips of the volunteers and assumed to be in equilibrium with arterial blood. Sampling of end-exhaled air was performed in conjunction with blood sampling post-exposure. All subjects lacked GSTT1 activity entirely, as determined by methyl chloride disappearance from blood erythrocytes *ex vivo*, and by polymerase chain reaction (PCR) genotyping.

3.1.2. Dichloromethane (Studies II, IV)

Dichloromethane (methylene chloride, DCM) is a solvent that is used abroad in many applications. Its largest use is as the principal active ingredient in organic-based paint strippers. It is used in both consumer and industrial paint removers. The second largest application of dichloromethane is in chemical processing. Due to its suspected carcinogenic properties, it is banned from use in Sweden, but is still used to a limited extent in the pharmaceutical industry (43). Present occupational exposure level limit value in Sweden is 35 ppm (short-term exposure 70 ppm).

Two data sets on dichloromethane exposure were used:

In the first study (96), 15 male subjects were exposed at up to 250-1,000 ppm at rest and light, moderate or heavy exercise according to four different exposure regimens. In all subjects, frequent sampling of end-exhaled air was performed during and up to 20 hours after the exposure. Frequent sampling of arterial blood was also performed during and up to 4 hours after exposure.

In the second study (32), 12 male lean and obese subjects were exposed to 750 ppm of dichloromethane at light exercise. Frequent sampling of end-exhaled air was performed during and up to 20 hours post-exposure. In six subjects, arterial blood was also sampled frequently during and up to four hours post-exposure. Sampling of subcutaneous fat was performed in all subjects at six time points up to six hours post-exposure.

3.1.3. Toluene (Study III)

Toluene is used in industry as a chemical intermediate and as a solvent. Its use is widespread, and workers using products containing toluene (*e.g.* painters) are likely to be occupationally exposed (97). Present occupational exposure level limit value in Sweden is 50 ppm (short-term exposure 100 ppm).

Six male subjects were exposed to 80 ppm of toluene at rest and light to heavy exercise (19). Frequent sampling of end-exhaled air was performed during and until 20 hours after the exposure. Frequent sampling of arterial blood and end-exhaled air was performed during and up to 2 hours after the exposure. Subcutaneous fat tissue was sampled up to six days post-exposure (20).

3.1.4. Styrene (Study V)

Styrene is used primarily as a monomer in the plastics industry for production of various polymers. Most occupational exposure occurs during production and processing of plastic products containing styrene (59). Present occupational exposure level limit value in Sweden is 20 ppm (short-term exposure 50 ppm). Data from three different studies were included:

Fifteen male subjects were exposed according to several regimens to up to 350 ppm of styrene at rest and various levels of workload (95). In all subjects, frequent sampling of end-exhaled air was performed during and up to 20 hours after the exposure. Frequent sampling of venous and arterial blood was also performed during and up to 20-60 minutes post-exposure.

Seven male subjects were exposed to styrene at 50 ppm during rest and light, moderate and heavy exercise (33). Frequent sampling of end-exhaled air was performed during and up to 20 hours post-exposure. In three subjects, arterial and venous blood was also sampled frequently during and up to four hours after exposure. Sampling of subcutaneous fat was performed in all subjects during one to fourteen days post-exposure.

One male and one female subject were exposed to styrene at 26, 77, 201, and 386 ppm during a workload of 50 W at four occasions (62). Arterialised capillary blood was sampled from a pre-warmed finger tip and analyzed for styrene during and up to three hours after each exposure

3.2. Structural models

In the first studies (I, II), a standard six-compartment model, encompassing compartments for working and resting muscle, lungs and arterial blood, well-perfused tissue, adipose tissue, and liver, was used.

As the six-compartment model failed to provide adequate predictions of toluene levels in adipose tissue (Study III), the fat compartment was split in two. The derived seven-compartment model was used in the subsequent studies (IV, V, depicted in Figure 2).

In the one study (V, styrene) where concentrations in venous blood were included in the analysis, a set of correction factors were introduced. In all studies, an artificial division of muscle tissue into compartments for "resting" and "working" muscle was made in the structural model. This was done in order to account for the increased perfusion of leg muscle tissue during exercise (48). Previously, observations in venous blood have been described as corresponding to washout from resting muscle tissue only (48), or as mixed washout from muscle and fat tissue (72). The antecubital venous blood sampling described in Study V is likely to also include some washout from the more perfused "working" muscle. In working muscle tissue, more blood is shunted. In order to account for the mixing of the wash-out blood from resting and working muscle compartments and the shunting of arterial blood occurring in working muscle, the venous blood samples were described in the model as corresponding to a mix of washout blood from resting muscle and shunted arterial blood. The correction factor describing the degree of mixing was regarded as an unknown model parameter.

Tissue distribution was assumed to be perfusion-limited in all studies.

For styrene, previous studies suggest that the inhalatory uptake is lower than predicted by reference values on alveolar ventilation, and that sampled end-exhaled air may not be a very accurate reflection of the amount of retained styrene (26, 47). One suggested explanation to this phenomenon is that significant amounts of styrene is desorbed from the lining if the lungs, a so-called "wash- in wash-out" effect. Thus, correction factors accounting for both the mixing between actual exhaled volatile and that desorbed in the linings of the lungs, and the reduction of the effective alveolar ventilation, were also introduced in the model.

3.3. Statistical model

The same statistical model was used in all studies, and is illustrated in Figure 4. The hierarchical model has two major components: the individual level and the population level. For each of the n_i subjects (i), concentrations of volatile (y) were measured experimentally at n_j time points (j). y is a matrix with dimensions i and j. The PBPK model (f) can predict the concentration-time profiles for an individual given its known exposure conditions (E), its unknown individual model parameters (θ), and known physiological covariates (φ) (*e.g.* body weight, oxygen uptake, etc) at a given point in time. There is a difference between the observed and the predicted concentrations due to assay error, possible model misspecification and random intra-individual variability in model parameters. This difference is accounted for by the error model. It was assumed that the errors were independent and lognormally distributed with a mean of zero and a variance of σ^2 on a logarithmic scale. The variance vector has up to four components ε_k, as the measurements in venous and arterial blood, end-exhaled air and subcutaneous fat

have different experimental protocols and are likely to have different precisions. The one exception to the assumption of lognormality is for ε_{fat} in study IV, where a normal distribution of ε_{fat} was assumed. In the analysis, ε is estimated along with the other model parameters.

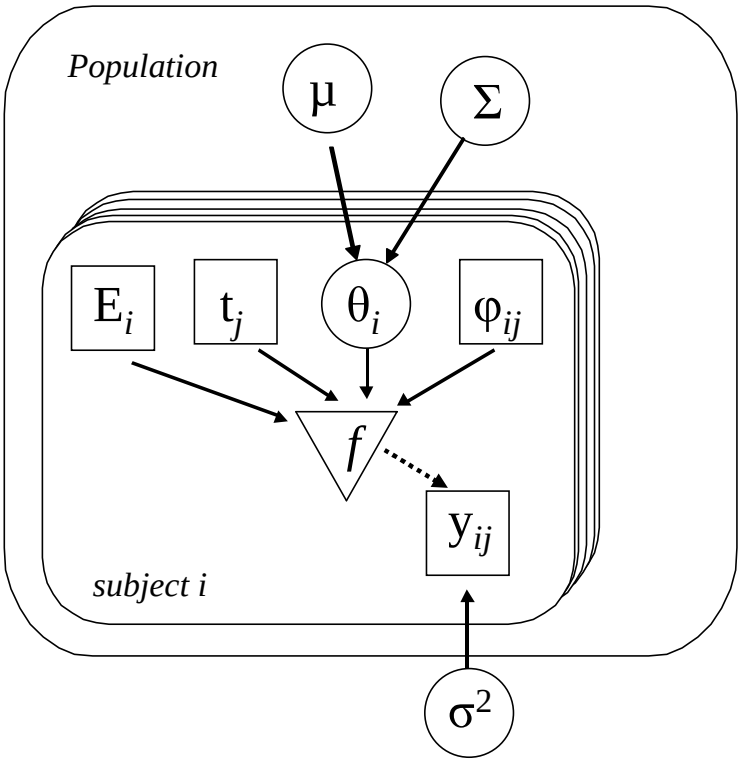

Figure 4. Graph of the statistical model describing the dependence relationships between variables. Symbols are: μ mean population parameters, Σ variances of the parameters in the population, *E* exposure conditions, *t* sampling times, θ unknown individual parameters, φ measured individual covariates, *f* toxicokinetic model, *y* measured methyl chloride concentrations in individual *i* at time *j*, and σ^2 variance of the experimental measurements. In the figure, each slice represents one individual subject.

At the population level, the inter-individual variability is described by assuming that the vector of unknown individual parameters $\theta = \{\theta_{i1}, \theta_{i2}, \ldots, \theta_{ini}\}$ is a sample from a lognormal population distribution with mean μ and matrix of scaled variances Σ. Both population parameters μ and Σ are affected by prior uncertainty.

Three types of nodes are featured in Figure 4: Square nodes represent variables for which the values are known by observation, such as *y* and φ, or fixed by the experimenters, such as *E* or *t*. Circle nodes represent unknown variables, such as θ or σ^2, which are estimated in the analysis. The triangle represents the deterministic PBPK model *f*. A plain arrow represents a direct statistical dependence between the variables of these nodes, while a dashed arrow represents a deterministic link.

3.4. Physiological parameters

Generally, the same parameterization was used in all studies in the thesis. However, as the fat compartment was split in two in the toluene study (III), and that model was adapted in subsequent studies as the standard model, the parameterization was slightly different in the first two studies (I, II). The actual parameterization shown in Table 1 refers to the later studies III-V. The reader is referred to the individual studies for the parameterization used in studies I and II.

In studies III, IV and V, the lean body mass was calculated from measured data (88, 89), as described in Table 1. To account for known physiological dependencies between some pharmacokinetic parameters, such as between lean body weight and organ volumes, dependent parameters were linked to body weight, height and workload via scaling functions (25, 30, 90). The scaling functions are also described in Table 1. The sum of all volume fractions add up to lean body weight minus skeleton, 13 percent of lean body weight. The muscle compartment included skin. The well-perfused compartment was calculated as the sum of brain, kidneys and other tissues (28). A density of 1.1 was assumed in all tissues (6), except for fat, for which density was assumed to be 0.92 (34).

The change in blood flow to various tissues with physical workload was considered independent of body size, and calculated with the assumption that the change is proportional to the excess oxygen uptake above rest (24). The equations used to describe the effect of physical workload (30) were derived according to suggested reference values (93), and are described in detail in Table 1.

The scaling functions used in the present studies have never been subject to any rigorous statistical validation. However, as they are generally accepted, we adapt the assumption of their validity until this research topic is explored further.

3.5. Prior distributions

In the early studies (I, II, III), prior distributions for the physiological parameters were derived using the available reference literature (17, 30, 36, 90, 93). The data sets in the earlier studies (I, II, III) were considered too sparse to yield information on some of the physiological parameters, and the model calibration in these studies was thus performed conditional on fixing of some parameters to their reference value.

In the later studies (IV, V), priors from previous Bayesian PBPK modeling efforts (II, III) were used in conjunction with substance-specific information on metabolic capacity and partitioning. This was done in accordance with the Bayesian approach to information gathering and updating of the current belief. In the last study (V), priors were used on all model parameters, except the compartment volumes, as only the product of the compartment volume and the

Table 1. Relationships between physiological variables in the PBPK models in III, IV and V.

Parameter	Mathematical relationship	Reference
Total body water (l, males)	$TBW = -12.86 + 0.1757 \cdot BHt + 0.331 \cdot BWt$	Watson *et al.*, 1980
Total body water (l, female)	$TBW = -2.097 + 0.1069 \cdot BHt + 0.2466 \cdot BWt$	Watson *et al.*, 1980
Fat free mass (kg)	$FFM = TBW / 0.72$	Widdowson, 1965
Lean body volume (l)	$BV = FFM / 1.1$	Behnke *et al.*, 1953
Volumes (l)		
Fat	$V_{fat} = (BWt - FFM) / 0.92$	Fidanza *et al.*, 1953
Lung + arterial blood	$V_{lung} = (0.00907 + 0.01933) \cdot BV$	Cowles, 1971
Liver	$V_{h} = 0.0285 \cdot BV$	Cowles, 1971
Working muscle	$V_{wm} = 0.344 \cdot BV$	Cowles, 1971
Resting muscle	$V_{rm} = 0.344 \cdot BV$	Cowles, 1971
Venous blood	$V_{blo} = 0.0832 \cdot BV$	Cowles, 1971
Well-perfused tissue	$V_{wp} = (0.0256 + 0.00532 + 0.0103) \cdot BV$	Cowles, 1971; Droz, 1992
Flows (l/min)		
Subcutaneous fat (males)	$Q_{scfat} = 0.5 \cdot V_{fat} \cdot QCf_{scfat}$	Fiserova-Bergerova, 1992
Subcutaneous fat (females)	$Q_{scfat} = 0.68 \cdot V_{fat} \cdot QCf_{scfat}$	Fiserova-Bergerova, 1992
Perirenal fat ($\Delta V_{O2}=0$)	$Q_{pfat} = 0.4 \cdot V_{fat} \cdot QCf_{pfat}$	Fiserova-Bergerova, 1992
Perirenal fat ($\Delta V_{O2}=0$, females)	$Q_{pfat} = 0.32 \cdot V_{fat} \cdot QCf_{pfat}$	Fiserova-Bergerova, 1992
Perirenal fat ($\Delta V_{O2}>0$, males)	$Q_{pfat} = QCf_{pfat} \cdot 0.4 \cdot V_{fat} + Q_{pfat}^{Work} \cdot 0.4 \cdot V_{fat} \cdot \Delta V_{O2}$	Fiserova-Bergerova, 1992
Perirenal fat ($\Delta V_{O2}>0$, females)	$Q_{pfat} = QCf_{pfat} \cdot 0.32 \cdot V_{fat} + Q_{pfat}^{Work} \cdot 0.32 \cdot V_{fat} \cdot \Delta V_{O2}$	Fiserova-Bergerova, 1992
Liver	$Q_{h} = QCf_{h} \cdot V_{h} \cdot (1 - QCf_{h}^{Work} \cdot \Delta V_{O2})$	Droz *et al.*, 1989a
Working muscle	$Q_{wm} = QCf_{m} \cdot V_{wm} + QCf_{wm}^{Work} \cdot V_{wm} \cdot \Delta V_{O2}$	Droz *et al.*, 1989a
Resting muscle	$Q_{rm} = QCf_{m} \cdot V_{rm}$	-
Well-perfused tissue	$Q_{wp} = QCf_{wp} \cdot V_{wp} \cdot (1 - QCf_{wp}^{Work} \cdot \Delta V_{O2})$	Droz *et al.*, 1989a
Cardiac output	$Q_{tot} = Q_{fat} + Q_{h} + Q_{wm} + Q_{rm} + Q_{wp}$	-

partition coefficient could be expected to be estimated with any precision (83), and the partition coefficients were deemed known with less precision.

Priors for the chemical-specific parameters were derived considering the previously published PBPK models, where available. When possible, *in vitro* values for humans were used to derive priors for the partition coefficients. In most cases, this amounted to the use of human *in vitro* values for blood: air partition coefficients and *in vitro* values from rat for the others. Likewise, for the metabolic parameters, previous estimates from earlier models were used, where available. Uncertainties were set accordingly.

For the population variances, priors were set using the available reference data, where available. For variances where reference data was available, uncertainties were set to small values, as the relatively small population samples in all studies were regarded as too sparse for any quantitative updating of the prior belief on population variance.

3.6. Bayesian computations

In all studies, Bayesian updating of the prior information on model parameters was performed via MCMC simulation using the Metropolis-Hastings algorithm. The software MCSim (14) was used in all studies, as it is the only software available that can perform Markov chain Monte Carlo simulation of complex, nonlinear models conveniently. The software Bugs (84) is also available for Bayesian population modeling, but is not convenient for complex non-linear systems such as PBPK models.

4. Results

The MCMC approach generally succeeded very well in deriving improved estimates of the population PBPK parameters while retaining physiologically plausible parameter values. An example of the simultaneous predictions derived using the individual posterior parameter estimates is given in Figure 5, where the fit of the model to styrene data from one subject from Study V is illustrated. This figure also serves an illustration of the level of richness that these experimental data sets provide.

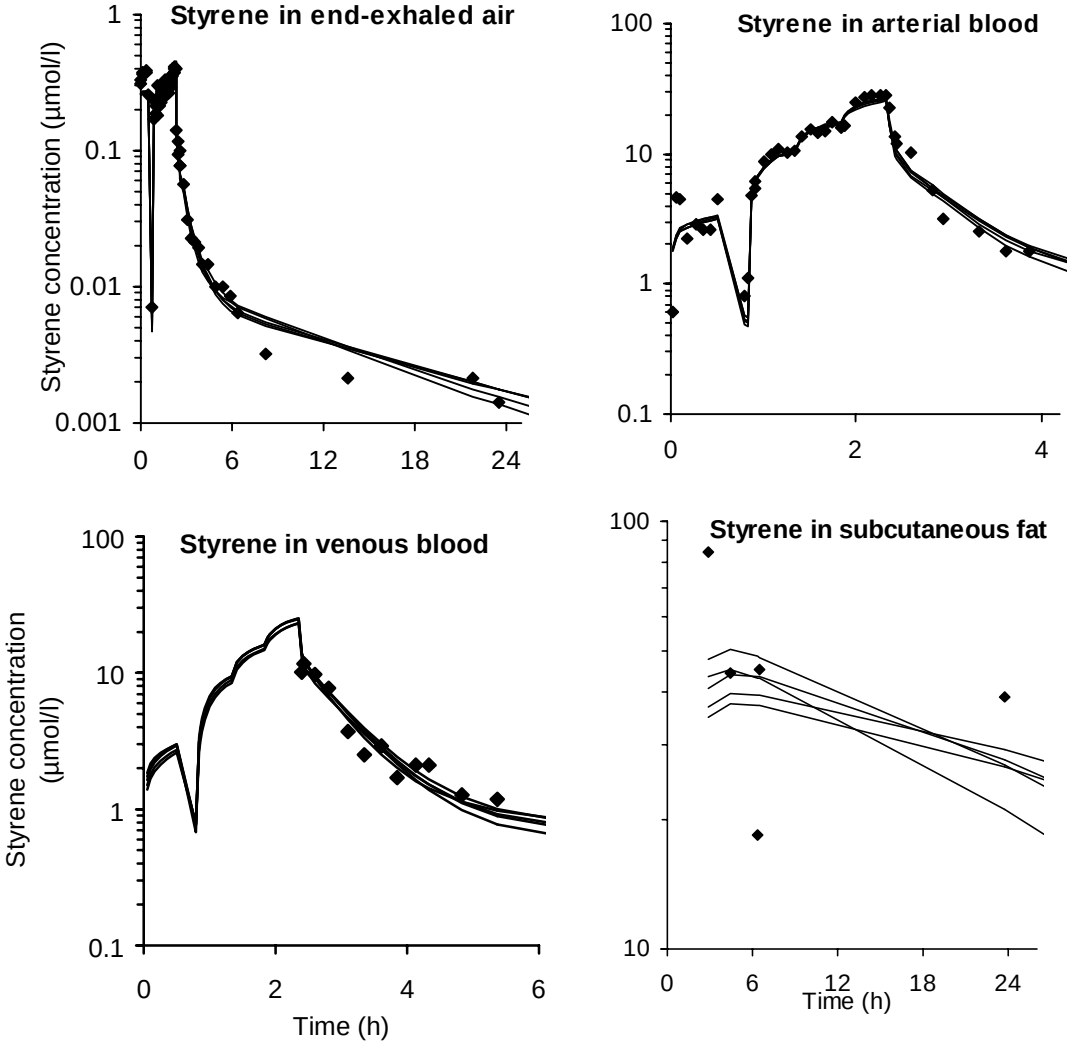

Figure 5. Example of fit of model to toxicokinetic data. Data from Study V. Observed (dots) and model-predicted (lines) concentration-time-profiles for styrene in one individual exposed to styrene at 50 ppm at rest, followed by an exposure-free interval, and then exposed again to 50 ppm of styrene at light, moderate and heavy exercise during three consecutive 30-minute intervals. Predictions were made using parameters from the last iteration of each run.

Table 2. Posterior estimates of the scaling coefficients for the physiological model parameters (given in studies I, II and in Table 3). SDs are given on a log scale.

Parameter	**Study I** (n=8)		**Study II** (n=5)		**Study III** (n=6)		**Study IV** (n=27)		**Study V** (n=24)	
	μ (SD)	Σ (SD)	**μ** (SD)	Σ (SD)	**μ** (SD)	Σ (SD)	**μ** (SD)	Σ (SD)	**μ** (SD)	Σ (SD)
Blood flows at rest (l/min/l tissue)										
Subcutaneous fat (QCf_{scfat})	**0.030**(1.10)	1.67(1.079)	**0.020**(1.09)	1.30(1.09)	**0.013**(1.16)	1.70(1.14)	**0.010**(1.11)	2.04(1.14)	**0.014**(1.11)	1.70(1.01)
Perirenal fat (QCf_{pfat})					**0.042**(1.10)	1.31(1.073)	**0.036**(1.081)	1.63(1.073)	**0.052**(1.069)	1.31(1.01)
Muscle (QCf_{m})	*n.e.*	*n.e.*	**0.028**(1.05)	1.27(1.01)	**0.024**(1.042)	1.11(1.01)	**0.028**(1.034)	1.14(1.01)	**0.028**(1.030)	1.14(1.01)
Liver (QCf_{h})	*n.e.*	*n.e.*	*n.e.*	*n.e.*	*n.e.*	*n.e.*	**1.1**(1.010)	1.10(1.01)	**1.1**(1.01)	1.10(1.01)
Well-perfused (QCf_{wp})	*n.e.*	*n.e.*	*n.e.*	*n.e.*	*n.e.*	*n.e.*	**1.2**(1.010)	1.10(1.01)	**1.21**(1.01)	1.10(1.01)
Perfusion change with exercise										
Fat(QCf_{pf}^{Work})	**0.019**(1.10)	1.15(1.085)	**0.014**(1.34)	1.55(1.24)	**0.071**(1.16)	1.33(1.094)	**0.037**(1.061)	1.52(1.094)	**0.058**(1.048)	1.33(1.01)
Working muscle (QCf_{wm}^{Work})	*n.e.*	*n.e.*	**7.5**(1.19)	1.21(1.13)	**8.4**(1.088)	1.13(1.047)	*n.e.*	*n.e.*	**8.5**(1.049)	1.17(1.01)
Liver (QCf_{h}^{Work})	*n.e.*	*n.e.*	**0.21**(1.10)	1.18(1.08)	*n.e.*	*n.e.*	*n.e.*	*n.e.*	**0.23**(1.085)	1.18(1.01)
Well-perfused (QCf_{wp}^{Work})	*n.e.*	*n.e.*	**0.15**(1.10)	1.15(1.07)	*n.e.*	*n.e.*	*n.e.*	*n.e.*	**0.18**(1.086)	1.15(1.0084)
Tissue : air Partition coefficients (% of prior estimate)										
Fat : Air (PC_{fat})	**85**(1.10)	1.10(1.01)	**55**(1.10)	1.10(1.01)	**88**(1.10)	1.15(1.01)	**63**(1.072)	1.16(1.01)	**82**(1.072)	1.16(1.01)
Muscle : Air (PC_{m})	**88**(1.11)	1.10(1.01)	**125**(1.07)	1.10(1.01)	**76**(1.064)	1.15(1.01)	**104**(1.038)	1.17(1.01)	**117**(1.038)	1.17(1.01)
Liver : Air (PC_{h})	**77**(1.10)	1.10(1.01)	*n.e.*	*n.e.*	**97**(1.091)	1.15(1.01)	**134**(1.14)	1.15(1.01)	**104**(1.14)	1.15(1.01)
Well-perfused : Air (PC_{wp})			*n.e.*	*n.e.*	**71**(1.079)	1.16(1.01)	**92**(1.10)	1.16(1.01)	**111**(1.047)	1.16(1.01)
Blood : Air (PC_{blood})	**102**(1.05)	1.05(1.036)	**109**(1.09)	1.40(1.10)	**87**(1.10)	1.29(1.12)	**116**(1.046)	1.30(1.10)	**140**(1.040)	1.30(1.10)
Intra-individual errors										
ε_{exh}	**1.49**		**1.24**		**1.37**		**1.21**		**1.27**	
ε_{art}	**1.29**		**1.16**		**1.16**		**1.22**		**1.26**	
ε_{ven}	*n.e.*		*n.e.*		*n.e.*		*n.e.*		**1.31**	
ε_{fat}	*n.e.*		*n.e.*		**1.66**		*a*		**1.74**	

[a]In this study, a normal distribution of ε_{fat} was assumed, and the estimate is thus not directly comparable. *n.e.* = not estimated.

The derived population PBPK parameters from the different studies are summarized in Table 2. In the table, the decreasing uncertainties around the population parameters through cumulative gathering of information in the Bayesian framework may be observed. As the studies progress, more parameters are estimated. The conclusions drawn from the individual studies are summarized below.

4.1. Modeling of data from non-conjugating subjects (Study I)

A PBPK model for methyl chloride was derived for the first time. The model generally provided good descriptions of the concentrations in exhaled air and arterialised blood, while maintaining biologically plausible parameter values. However, there were some deviations between model and data. The observed time course in blood followed a three-phase decay pattern after exposure, whereas in end-exhaled air, at least four phases could be discerned. A standard PBPK model for respiratory uptake was used, where the observations in end-exhaled air were assumed to be an accurate reflection of the simultaneous time course in arterial blood. The changing ratio of simultaneous concentrations in end-exhaled air and arterial blood seemed to challenge this common PBPK model assumption. The posterior estimates of model parameters produced a slight over-prediction of the concentrations in end-exhaled air between 30 and 180 minutes post-exposure (Figure 6). As may be gathered from Figure 6, the predicted approach to steady state was found to be faster than in the observed kinetic data and an over-prediction of the concentrations in blood at the latest time points could also be noted.

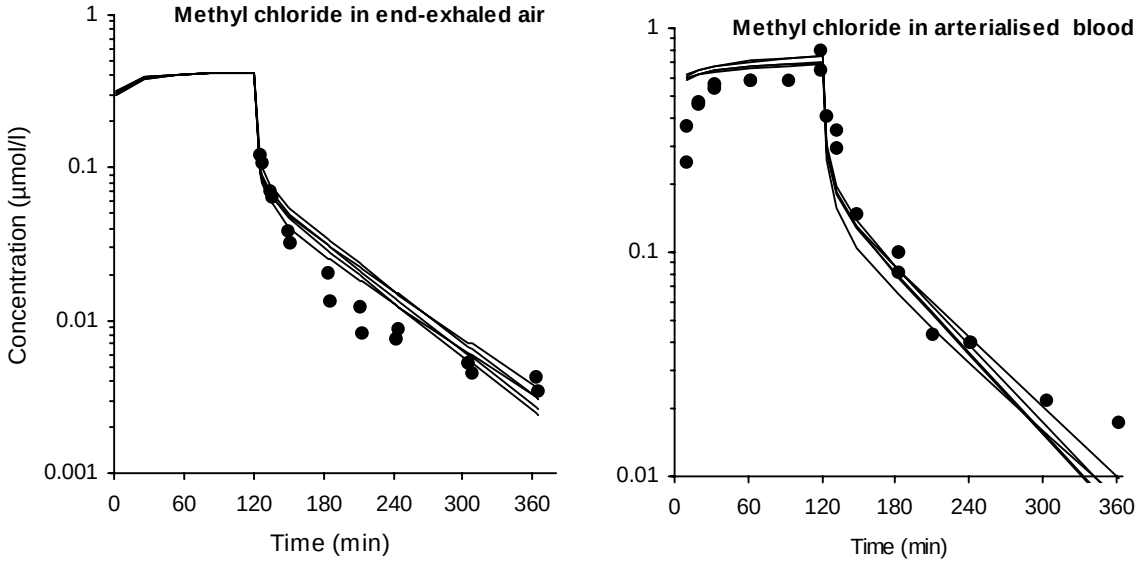

Figure 6. Observed (dots) and predicted (lines) concentration-time-profiles for methyl chloride in exhaled air and arterialised blood for one representative individual. Data from Study I. Predictions were made using the last iteration of the last Markov chain being run in the analysis.

4.2. The effect of physical exercise on the kinetics of dichloromethane (Study II)

A population PBPK model for dichloromethane that accounted for the effect of physical exercise on tissue perfusions was derived. The equations for intra-individual variability in conjunction with exercise developed by Droz and co-workers (30) were calibrated against kinetic data from five subjects exposed to dichloromethane at rest and light, moderate and heavy exercise. The equations were found to provide a good description of the intra-individual variability in conjunction with temporal changes in exercise levels. One exception was the parameters describing the changing perfusion of fat tissue. The derived posterior estimate of the exercise-related increase in fat perfusion was also lower than indicated by the prior information. In addition, the parameters governing the disposition in fat tissue were associated with larger posterior uncertainties than those for the disposition in other tissue groups, as may be seen in Table 2.

A Bayesian estimate of the population distribution of the parameters for the dominating metabolic pathway for dichloromethane was derived. The estimate was thus a compromise between the information from *in vitro* data and the toxicokinetic information present in the experimental data. This represents the first estimate of dichloromethane kinetics that has been validated against any *in vivo* human data on the individual level.

4.3. The kinetics of toluene in subcutaneous fat (Study III)

A population PBPK model for toluene was derived. In order to describe the experimental observations in subcutaneous fat accurately, the fat compartment was split in two. The model was then calibrated against the simultaneous time courses of toluene in arterial blood, exhaled breath, and subcutaneous fat in individuals exposed to toluene. The increased perfusion of perirenal fat associated with physical workload was best described if it was set to the same, elevated, level during all exercise levels, rather than scaled directly to the increase in oxygen uptake as suggested previously (28, 30). No increase in subcutaneous fat perfusion during exercise could be detected.

This represents the first reliable estimate of the population kinetics of toluene.

4.4. Risk assessment of dichloromethane exposure (Study IV)

The previously developed population PBPK model for dichloromethane (Study II) was modified according to the findings in Study III. The model was then fitted to extensive human toxicokinetic data from 27 male volunteers, including the data from the five volunteers used in Study II. The precisions of the estimated population PBPK model parameters were improved compared with the previous modeling effort (Study II, as may be observed in Table 2).

In a second step, excess cancer risks according to an existing cancer risk model (21) in conjunction with lifelong exposure to 1-1,000 ppm of dichloromethane

were estimated by regular Monte Carlo simulation. As dichloromethane cancer risk has been linked to metabolic activation via glutathione transferase T1, data on the frequencies of this gene in the Swedish population were incorporated into the simulations. The derived population estimates are illustrated in Figure 7. Estimated mean and median excess risks were in general agreement with those derived previously (31). The estimated confidence bounds are also illustrated numerically in Table 3, where they are juxtaposed with the previous estimates. However, as the estimates in IV are based on a PBPK model that has been calibrated against extensive human data using a population model, they are subject to much larger reliability than the previous estimates.

4.5. Population modeling of styrene (Study V)

A population PBPK model for styrene was derived according to the findings in Study III and the population kinetics of styrene were assessed in a rigorous statistical analysis for the first time. The uncertainties around the partition coefficients and metabolic parameters for were reduced considerably. In addition to the data from one subject illustrated in Figure 5, the concentration-time curves in arterialised blood for one male and one female subject exposed to styrene at four occasions are illustrated in Figure 8.

The earlier problem with a changing ratio of simultaneous concentrations in end-exhaled air and arterial blood (Study I) was solved by a reduction of the alveolar ventilation to a value lower than the physiological value in a manner similar to what had been suggested previously for rodents (26, 47). The concentrations in venous blood were found to be a poor reflection of the uptake of styrene and an unreliable descriptor of the kinetics of styrene compared with arterial or arterialised blood.

The intra-individual perfusion of fat tissue was found to be very difficult to describe accurately in a PBPK model using the present knowledge, and was detected as a possible source of model uncertainty.

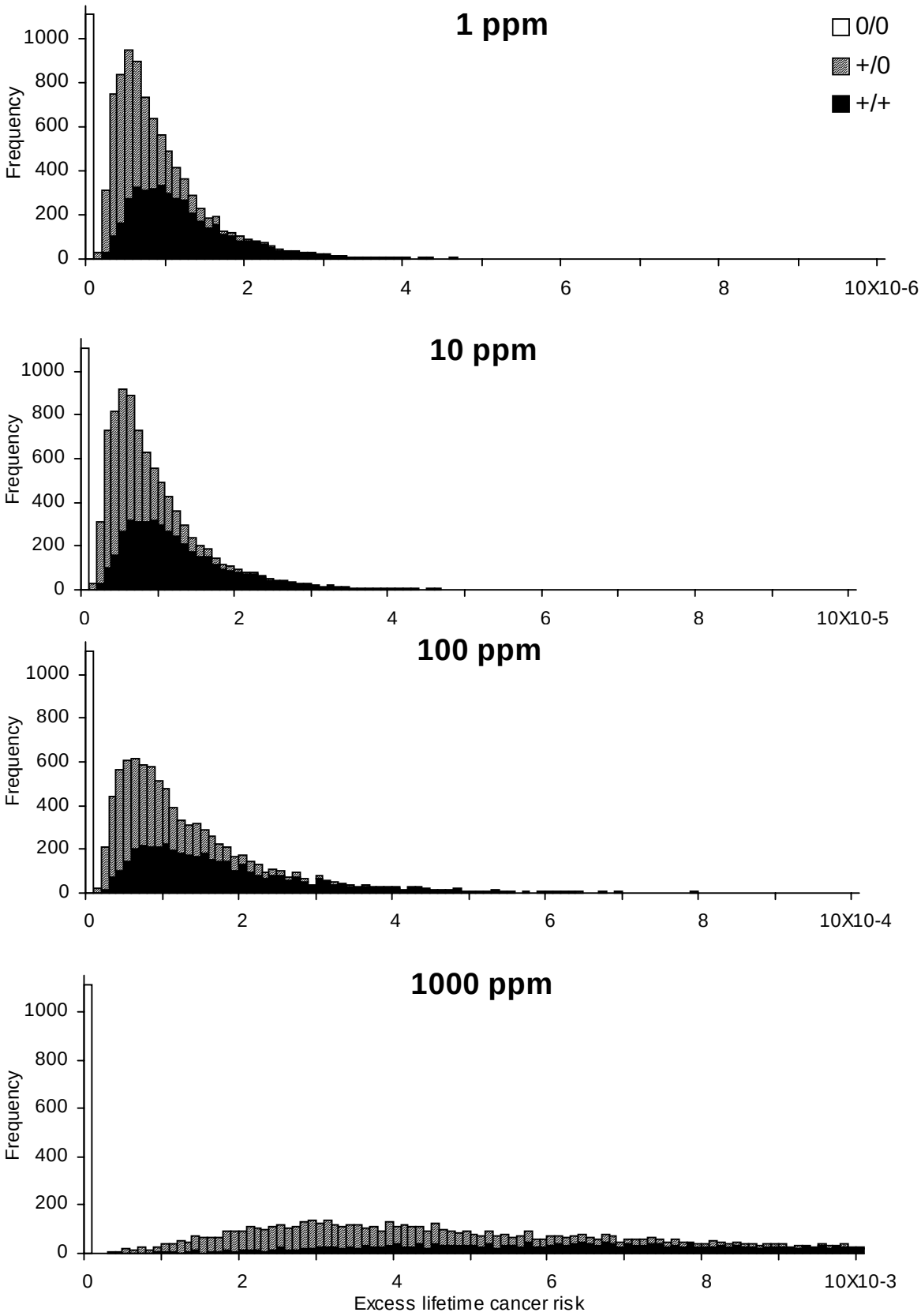

Figure 7. The model-predicted distribution of risks to humans after life-long exposure to concentrations of 1-1000 ppm of dichloromethane. Taken from Study IV. The predictions are grouped according to genotype, as indicated.

Table 3. Risk estimates for Dichloromethane at various levels of life-long exposure. Taken from IV.

Exposure level	Group	Mean risk	Upper confidence limit[a]			
			50%	95%	99%	99.9%
1000 ppm	0/0[b]	0	0	0	0	0
	+/0[b]	$45 \cdot 10^{-4}$	$37 \cdot 10^{-4}$	$100 \cdot 10^{-4}$	$170 \cdot 10^{-4}$	$370 \cdot 10^{-4}$
	+/+[b]	$130 \cdot 10^{-4}$	$89 \cdot 10^{-4}$	$370 \cdot 10^{-4}$	$730 \cdot 10^{-4}$	$1200 \cdot 10^{-4}$
	Swedish population[b]	$76 \cdot 10^{-4}$	$47 \cdot 10^{-4}$	$240 \cdot 10^{-4}$	$540 \cdot 10^{-4}$	$1100 \cdot 10^{-4}$
	American population[c]	*$14 \cdot 10^{-4}$*	*$13 \cdot 10^{-4}$*	*$360 \cdot 10^{-4}$*	*n.d.*	*$90 \cdot 10^{-4}$*[d]
100 ppm	0/0[b]	0	0	0	0	0
	+/0[b]	$9.0 \cdot 10^{-5}$	$7.1 \cdot 10^{-5}$	$22 \cdot 10^{-5}$	$34 \cdot 10^{-5}$	$54 \cdot 10^{-5}$
	+/+[b]	$18 \cdot 10^{-5}$	$14 \cdot 10^{-5}$	$44 \cdot 10^{-5}$	$69 \cdot 10^{-5}$	$100 \cdot 10^{-5}$
	Swedish population[b]	$12 \cdot 10^{-5}$	$8.5 \cdot 10^{-5}$	$34 \cdot 10^{-5}$	$56 \cdot 10^{-5}$	$100 \cdot 10^{-5}$
	American population[c]	*$8.1 \cdot 10^{-5}$*	*$7.8 \cdot 10^{-5}$*	*$20 \cdot 10^{-5}$*	*n.d.*	*$76 \cdot 10^{-5}$*[d]
10 ppm	0/0[b]	0	0	0	0	0
	+/0[b]	$6.1 \cdot 10^{-6}$	$5.1 \cdot 10^{-6}$	$14 \cdot 10^{-6}$	$22 \cdot 10^{-6}$	$33 \cdot 10^{-6}$
	+/+[b]	$12 \cdot 10^{-6}$	$10 \cdot 10^{-6}$	$27 \cdot 10^{-6}$	$42 \cdot 10^{-6}$	$64 \cdot 10^{-6}$
	Swedish population[b]	$8.0 \cdot 10^{-6}$	$6.2 \cdot 10^{-6}$	$22 \cdot 10^{-6}$	$34 \cdot 10^{-6}$	$60 \cdot 10^{-6}$
	American population[c]	*$7.7 \cdot 10^{-6}$*	*$7.0 \cdot 10^{-6}$*	*$20 \cdot 10^{-6}$*	*n.d.*	*$42 \cdot 10^{-6}$*[d]
1 ppm	0/0[b]	0	0	0	0	0
	+/0[b]	$5.9 \cdot 10^{-7}$	$5.0 \cdot 10^{-7}$	$13 \cdot 10^{-7}$	$21 \cdot 10^{-7}$	$31 \cdot 10^{-7}$
	+/+[b]	$12 \cdot 10^{-7}$	$9.9 \cdot 10^{-7}$	$26 \cdot 10^{-7}$	$40 \cdot 10^{-7}$	$62 \cdot 10^{-7}$
	Swedish population[b]	$7.8 \cdot 10^{-7}$	$6.1 \cdot 10^{-7}$	$21 \cdot 10^{-7}$	$33 \cdot 10^{-7}$	$57 \cdot 10^{-7}$
	American population[c]	*$7.1 \cdot 10^{-7}$*	*$6.6 \cdot 10^{-7}$*	*$17 \cdot 10^{-7}$*	*n.d.*	*$31 \cdot 10^{-7}$*[d]

[a]The percentage states the fraction of subjects in the population who are at a risk equal to or less than this limit value.
[b]Study IV.
[c]Values from El-Masri *et al*. (1999) n.d= Not determined.
[d]Given as maximum risk by El-Masri *et al*. (1999).

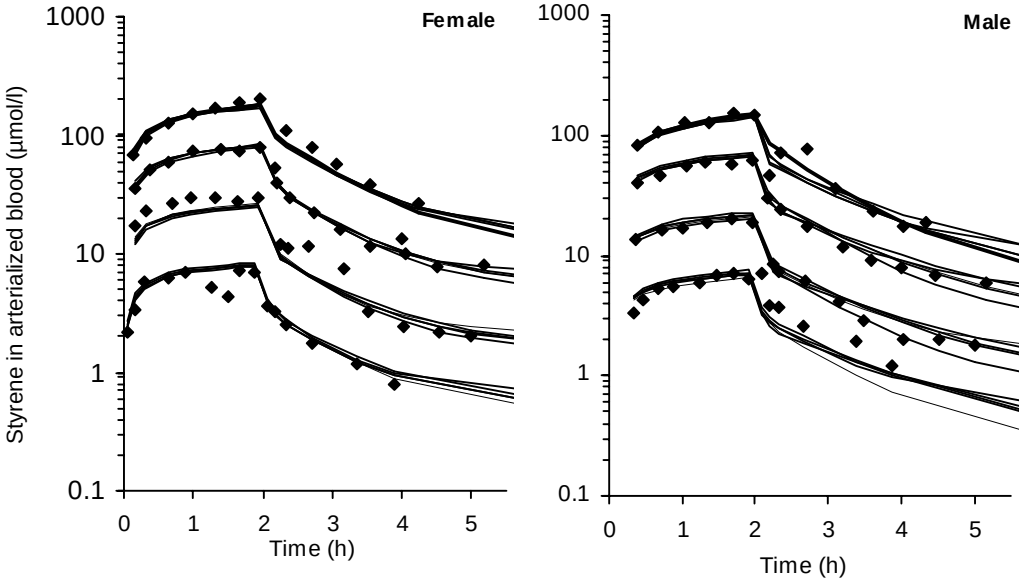

Figure 8. The model-predicted concentration-time curves in arterialised blood for one male and one female subject exposed to styrene at 26, 77, 201, and 386 ppm during a workload of 50 W at four occasions. Study V. Predictions were made using the last iteration of the last Markov chain being run in the analysis.

5. Discussion

The Bayesian population approach was applied to PBPK modeling of toxicokinetic data sets on methyl chloride, toluene styrene and dichloromethane using MCMC simulation. The results provide an extension and application of techniques developed by Bois and co-workers during recent years (10-13, 15). Information was gathered on the population variability in the kinetics of the volatiles under study. This information may be useful in risk assessment, as illustrated for dichloromethane in Study IV.

5.1. Metabolic parameters

Dichloromethane metabolism takes place via two competing pathways: Metabolism by mixed-function oxidases (MFO) (1), and by glutathione-*S*-transferase T1 (GSTT1) (55). The GSTT1 pathway is assumed to be the one associated with an increase in cancer risk (2). However, at low exposure levels, the MFO pathway is dominating (71). At low exposure levels, the inter-individual variability in total metabolism mainly reflects variability in the MFO pathway. Thus, the most sensitive individuals in Study IV possess a combination of high GSTT1 activity and a low metabolic capacity for the protective MFO pathway, for which a large population standard deviation (SD) was estimated.

The MFO pathway is most likely mediated by cytochrome P450 2E1 (Cyp2E1). Cyp2E1 is known to be highly inducible, which is a likely explanation of the considerable inter-individual variability of this pathway. Cyp2E1 is also believed to be primarily responsible for the metabolism of various other volatiles (75), including styrene and toluene. The posterior population SDs for the maximum metabolic capacities for toluene (Study III), dichloromethane (Study IV), and styrene (Study V) are very similar (1.71, 1.69, and 1.66, on a log scale, respectively). Previously, Caucasians and Orientals have been shown to exhibit considerable differences in metabolic capacity for styrene (44). In addition, much attention has been given to the possible influence of genotype (70) and other covariates such as alcohol consumption and smoker status (57) on Cyp2E1 metabolic capacity. Studies of possible relationships have been conducted on humans both *in vivo* and *in vitro*, but the results have so far not been conclusive so far with regards to the identification of significant covariates. A toxicokinetic study of the influence of various covariates (e.g. alcohol consumption, genotype, etc) on the individual Cyp2E1 metabolic capacities using mixed effects modeling (64, 82) would be of help in explaining the considerable estimated random variability of *in vivo* metabolic capacity in studies III, IV and V. In this manner, sensitive groups could also be identified. Unfortunately, data on very few covariates were collected when the original studies (19, 32, 33, 95, 96) were undertaken. In addition, all studied subjects were of the same ethnicity, and

relatively homogenous with regards to age, sex and body build. Experimental studies of the kinetics of these chemicals in a more heterogeneous population should contribute much valuable information on any covariate effects. This would allow for a more precise estimation of the population variability in toxicokinetics than offered by GSTT1 genotyping or phenotyping only.

5.2. Respiratory uptake

In the early studies (I-IV), as in any standard PBPK model, the concentration in end-exhaled air was assumed to be equal to the concentration in arterial blood, divided by the blood: air partition coefficient. If the generally accepted theories underlying gas exchange in the model (flow-limitation, instantaneous equilibrium, constant value of blood: air partition coefficient) were true, the relationship between concentrations in end-exhaled air and arterial blood should be proportional. These standard assumptions on inhalation uptake were challenged by our results when fitting simultaneous concentration-time curves in blood and end-exhaled air (studies I and III). In these studies, we found that standard PBPK models under-predicted the concentrations in end-exhaled air during exposure at rest and over-predicted those during exposure at increasing exercise levels. Meanwhile, the descriptions of the simultaneous concentrations in blood were excellent. Non-constant simultaneous ratios of concentrations in end-exhaled air and arterial blood can also be observed by closely scrutinizing data from similar published inhalation experiments, *e.g.* (96), (figure 7, dichloromethane), (94), (figure 8, toluene).

In Study V, this problem was solved by a reduction of the alveolar ventilation to a value lower than the physiological value in a manner similar to what had been suggested previously for rats (26, 47) when describing the wash-in wash-out effect in the upper respiratory airways. The reduction of the alveolar ventilation affected the estimated values of the blood: air partition coefficient, as well as the metabolic parameters. The validity of the assumption of an instant equilibrium between arterial blood and inhaled air can only be tested by simultaneous monitoring of the concentrations in blood and exhaled air during exposure. This is currently not standard practice when toxicokinetic data are collected for subsequent use in calibration of PBPK models.

If the commonly used assumption of instantaneous equilibrium in the inhalation uptake of volatiles is not valid in all cases, this is a fundamental model misspecification that is likely to weaken the conclusions generally drawn from many inhalation PBPK models. Misspecification of the inhalation uptake and elimination via exhalation may have a profound effect on the estimated metabolic capacity, and ultimately, of target dose. Preferably, this assumption should be challenged in order to ensure a proper description of the respiratory uptake in PBPK modeling.

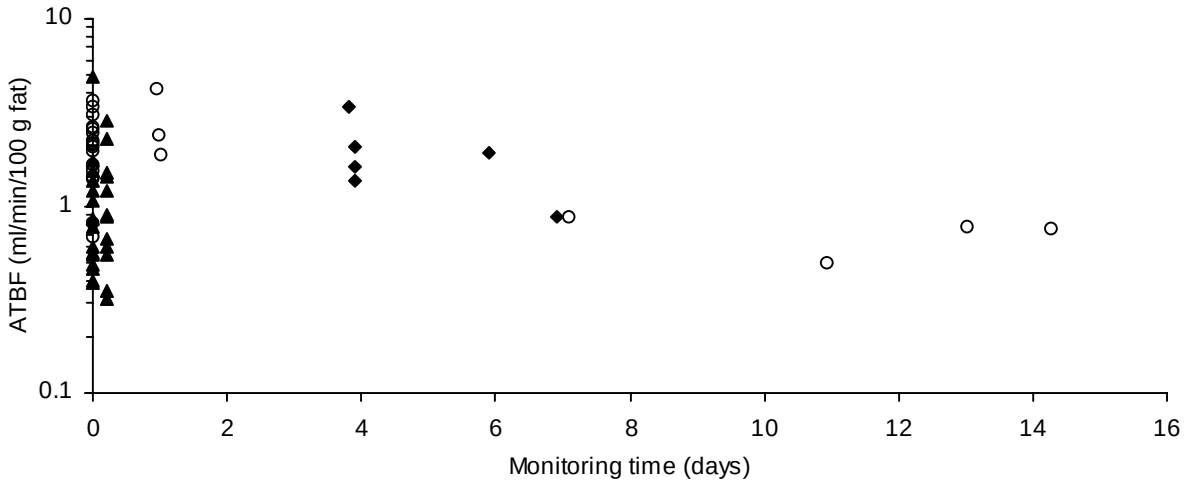

Figure 9. Estimated individual subcutaneous adipose tissue blood flows (ATBF) in studies of toluene (Study III, diamonds), dichloromethane (Study IV, triangles) and styrene (Study V, circles) plotted against monitoring time in subcutaneous fat tissue.

5.3. Perfusion of subcutaneous fat

In PBPK models, the fat content in the body is normally accounted for by a single compartment. Distribution in fat tissue, as in any other compartment, is assumed to be perfusion-limited. The observation that the time course in sampled subcutaneous fat tissue can not be described accurately using a single, perfusion-limited fat compartment (Study III) seems to challenge the standard approach to modeling of the kinetics in fat tissue. The approach is challenged further in Figure 9, where the individual estimates of subcutaneous fat perfusion from studies III, IV and V are plotted against the monitoring time. As seen in the figure, in subjects where fat tissue was monitored for a longer time than one day, the posterior estimates of the subcutaneous fat tissue perfusion decreased with increasing monitoring time. In addition, the posterior estimates of the fat: air partition coefficients in all studies were 12 to 45 per cent lower than the prior estimates (Table 2). The prior estimates were based on *in vitro* data from rat. Meanwhile, for the other partition coefficients, no tendencies can be detected among the posterior estimates.

Reference data on adipose tissue blood flow, as summarized in reviews of tissue perfusion (16, 90), indicate a large inter-individual variability in adipose tissue blood flow. In addition, body fat includes two kinds of adipose tissue: perirenal, ("inner") fat and subcutaneous fat. A division of fat tissue into richly and poorly perfused fractions has previously been found to provide much better descriptions of the concentration-time curves when PBPK models for anesthetics have been developed (35, 36). This approach was adapted in the studies III, IV and V. However, the local perfusion of fat tissue has been found to be inversely proportional to the thickness of the fat layer (68). The vascularisation of fat tissue is apparently decreasing linearly as the outer layers of fat tissue are reached. In reality, there is thus a range of unit perfusions distributed across the fat compartment. There is also a large uncertainty around the measured fat tissue

perfusions due to measurement error and intra-individual and inter-regional variability (69).

The perfusion of fat tissue is estimated with greater precision in subjects with extensive monitoring of fat tissue (Study V). As may be seen in Figure 9, the estimated fat perfusions in these subjects are low. The portions of fat tissue being sampled apparently correspond to the least perfused, outermost portions of fat tissue. As fat perfusion was *a priori* known to be subject to large intra- and inter-individual variability, much freedom was allowed when estimating the fat tissue perfusions in the individual subjects. As concentrations in fat tissue are difficult to measure with precision, as discussed in studies III, IV and V, the estimated perfusions of the two fat tissue compartments for subjects with short monitoring time in subcutaneous fat were dictated by the concentration-time curves in blood and exhaled air, rather than those in subcutaneous fat. However, as monitoring time in subcutaneous fat increased, the time courses in fat tissue became more influential on the estimated subcutaneous fat perfusion. The observation that the posterior estimates of the fat: air partition coefficients are lower than estimated for rat *in vivo* is another indication that the assumption of an even distribution throughout the fat compartments may not be valid.

Theoretically, the problem of sampling-dependent estimation of fat tissue perfusion may be solved by the use of an additional, third, fat compartment. However, this would render the model less based in measured physiological data. Another, probably more attractive, solution would instead be to incorporate the previous finding that the local fat tissue perfusion decreased with increasing thickness of the fat tissue layer into the model. The most accurate model would probably be one that incorporated a gradual decrease of the perfusion of fat tissue across the compartment volume. Such a model would not be the equivalent of the standard diffusion-limited models that have been used in PBPK models. The standard models for diffusion-limitation in PBPK modeling usually incorporate an artificial division of the compartment in two, rather than make use of a range of unit perfusions across the fat compartment to describe the diffusion-limitation. Rather, a model resembling the “parallel tube” model that has been suggested for the description of liver perfusion (4) may be needed.

A proper characterization of fat tissue perfusion is important when the metabolic parameters are assessed from kinetic data. A distribution of chemical according to other patterns than the usually assumed, perfusion-limited behavior would be a possible confounder of the estimated metabolism, and could possibly lead to erroneous estimates of target dose. There is thus a need for the development of models that address these issues.

These possible misspecifications in the standard PBPK model descriptions of fat tissue perfusion could be detected in the present thesis as PBPK model calibration against simultaneous measured concentrations in exhaled air, blood, and fat tissue was performed for the first time in humans.

5.4. Modeling of the change in fat perfusion with exercise

A proper description of the magnitude and time course of the changes in fat tissue perfusion with workload is important when applying PBPK models for lipophilic substances. However, so far there have been few attempts to include such descriptions when modeling exercise, and this issue has not been addressed at all in published reviews of reference values for physiological parameters for use in kinetic models (16, 90). When the change in fat perfusion is included in PBPK models, the perfusion of fat tissue is usually assumed to be proportional to the oxygen uptake. However, this assumption has never been challenged against any toxicokinetic data previously. In the studies of the kinetics of toluene in fat tissue, the best description of the kinetic data was obtained when no increase in the perfusion of subcutaneous fat with exercise was allowed. The increased perfusion of perirenal fat was set to a constant level during all exercise levels (Study III). This approach to temporal changes in fat perfusion was adapted as the standard in subsequent studies (IV, V).

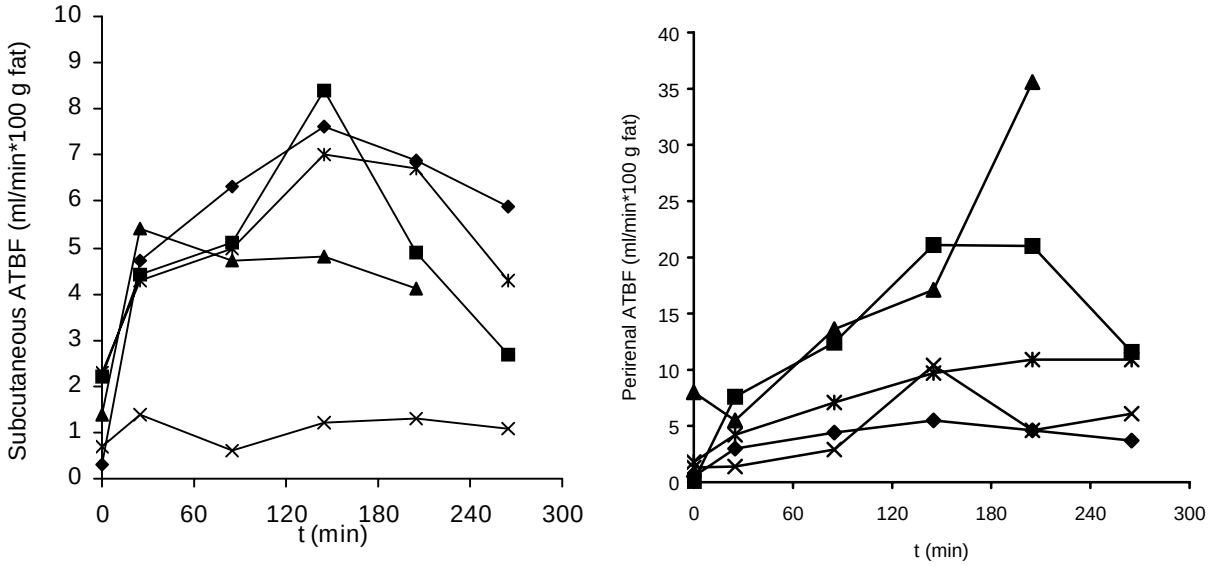

Figure 10. Individual subcutaneous and perirenal adipose tissue blood flows measured simultaneously before, during and after four 50-minute periods of exercise at a constant workload of 120 W. The figure (adapted from Study III) was derived using data taken from Bülow and Madsen (1978), table 1.

The only available reference values for the perfusion of fat tissue during exercise are those suggested by Åstrand (93). The Åstrand reference values for fat perfusion at rest and at 50, 100, and 150 W of exercise were based on two experimental studies (17, 18). In these studies, Bülow and Madsen measured the unit perfusion of fat tissue during prolonged exercise at a workload of 120 W. In the first of these studies (18), only subcutaneous fat tissue was monitored, and no individual perfusions were reported. In the second study (17), both perirenal and subcutaneous fat were monitored, and individual perfusions were reported for all

five subjects in the study. As the results were presented only in a tabular format in the original publication, they are plotted in Figure 10, which is adapted from Study III. The fat perfusions do not stabilize until two to three hours of exercise at a constant workload has passed. In addition, there is a large inter-individual variability. In later studies, the reference values from Åstrand were incorporated into an algorithm describing the perfusion of fat tissue at different workloads (28, 30). No validation of this algorithm against any kinetic data was done, as the equation was employed to summarize available reference data for subsequent use in simulations only.

When the very slow observed approach to steady state during prolonged, heavy exercise (Figure 10) is considered, our model for changes in fat perfusion during relatively short periods of exercise, is very likely. The circumstance that fat perfusion during exercise is best described if it is not set proportional to oxygen uptake is a new finding. This finding should preferably be taken into account when modeling the pharmacokinetic behavior of lipophilic substances during exercise.

Unfortunately, Bülow and Madsen restricted the monitoring of the post-exercise fat perfusion to one measurement in each subject. The most physiologically realistic description of the decreasing fat tissue perfusion post-exposure might be a slow, linear decrease. However, to our knowledge, there are no data in the physiological literature on the magnitude, slope or inter-individual variability of this decrease. It would indeed be possible to derive an empirical model for the slow increase and decrease of fat tissue perfusion. However, such a model would not be based in any external physiological data, and thus less convincing for inference to the general population. In any case, the issue of how to account for the changing perfusion of fat tissue accurately remains uncertain. Clearly, more experimental work in this area is needed.

5.5. Intra-individual variability in other model parameters

In the PBPK modeling studies, the suggested equations for the intra-individual variability in tissue perfusions were calibrated against toxicokinetic data for the first time. However, there are also other possible sources of intra-individual variability that might influence the performance of PBPK models. An increase in the blood: air partition coefficient in humans after a meal has been observed for several volatiles, including styrene and toluene (27), but also halothane, methylene chloride, and metoxyflurane (37), with larger deviations for the more lipophilic volatiles. Such variability may contribute to the possible intra-individual variability in elimination via exhalation, as the elimination via exhalation is largely governed by the blood: air partition coefficient. Judging from studies of the intra-individual variability in partition coefficients among rats (51), this variability may very well be of importance with regards to a proper quantification of the metabolic capacity, although not of any quantitative importance in terms of uptake or inhalation.

In Study I, all elimination occurred by exhalation. The level of physical exercise post-exposure was not standardized in the experimental study, as the subjects were allowed to move freely. As the alveolar ventilation is correlated to the level of exercise, and elimination is also governed by alveolar ventilation, intra-individual variability in exercise level has a direct effect on the total elimination in the subjects in Study I. In that study, the post-exposure alveolar ventilation was approximated in the model as being constant and corresponding to an activity level of 25 W. The true magnitude of the intra-individual variability in physical activity is unknown, but is likely to vary over time between levels corresponding to a range of 0 - 50 W, and may then be subject to large fluctuations over time.

In Study V, data from two subjects exposed to styrene on four separate occasions (62) were modeled. The observed and model-predicted concentration-time curves are depicted in Figure 8. In the model, the metabolic capacities were assumed to be constant over time. In addition, the pulmonary and alveolar ventilations were assumed to be constant at a given level of workload. As may be gathered from Figure 8, the model seems to slightly over-predict the concentrations at some occasions and under-predict the concentrations at other occasions. The deviations are not large, but noticeable and systematic. This variability may be modeled as inter-occasion variability (52, 61), but the source of this variability is uncertain. It could be enzyme induction, but intra-individual variability in ventilation and/or the blood: air partition coefficient may also contribute. It would be possible to integrate interoccasion variability in the present Bayesian population PBPK framework. In fact, according to Bernillon and Bois (7), it has already been done (preliminary data).

5.6. Sensitivity analysis in Bayesian modeling

In the thesis, MCMC simulation was combined with a hierarchical model. The use of a population model allowed us to separate inter- and intra-individual variability. In addition to the ability of discriminating variability from uncertainty and to providing improved estimates of uncertainty for model predictions, PBPK/MCMC coupling also helped us with model checking. As the MCSim software has now been used on several data sets, we have a reasonable assurance that the deviations we encountered in fitting our kinetic data are due to intra-individual variability and/or model misspecification rather than inadequate calibration or inappropriate choice of priors. Still, the issue of the sensitivity of the results to the choice of priors is an interesting topic. As already stated in the Introduction, a Bayesian approach allows for incorporation of much subjectivity into the analysis. During the development of the models, the effect of changing the prior distributions were tested continually, as in any model development, but as the MCSim software, as well as the entire approach, is fairly new, there are no formalized procedures to sensitivity analysis available. Thus, no such formalized sensitivity analysis was performed. In addition, the complexity of PBPK models renders the development of formalized procedures for sensitivity analysis a difficult task. This is a limitation of the MCMC technique, but in that respect, it certainly much better

than the currently practiced sensitivity analysis of frequentist-based PBPK models (42). That technique amounts to no more than an investigation of the influence of a certain, isolated model parameter on model predictions over time, conditional on the location of the other parameters. In a sense, the frequentist-based sensitivity analysis may be regarded as a primitive version of MCMC sampling. In MCMC, the likelihood of the data given a certain parameter is instead tested automatically during the calibration procedure. Ideally, a rigorous, formalized, sensitivity analysis should be performed also in MCMC/PBPK modeling. Such a sensitivity analysis should incorporate an assessment of the validity of the assumed population distributions, among other model features such as the question of diffusion-limited versus perfusion-limited tissue perfusion, etc. Non-parametric approaches have already been suggested in order to assess population distributions in a fully Bayesian context (79, 86). However, the computational burden associated with MCMC/PBPK modeling precludes the rigorous testing of many alternative models or the application of non-parametric approaches, especially when complex models such as PBPK are calibrated against very rich data from relatively few subjects. The only available choice may in many cases be to make a number of assumptions, and rely on these assumptions without any formal testing of their validity.

5.7. The Bayesian approach in risk assessment

Study IV has some general implications for noncancer risk assessment. An uncertainty factor of 10 is often used for noncancer risk assessment to account for inter-individual differences between humans in uptake and disposition of the chemical (toxicokinetics) and sensitivity of the target organ (toxicodynamics). This factor is commonly subdivided in two equal factors of 3.16 to account for variability in kinetics and dynamics, respectively. In a merged analysis of toxicokinetic data for 60 substances (78) it was concluded that for kinetics, the percentage of individuals not covered by a factor of 3.16 away from the mean was 0.07 per cent, assuming normal distribution and 0.9 per cent assuming lognormal distribution. In Study IV, per cent of the individuals in the Swedish population were estimated to not be covered by a factor of 2.7-3.2 away from the mean (Table 3). These results supports the point of caution already pointed out that a higher intra-species uncertainty factor for kinetics than 3.16 should be considered for substances which, like dichloromethane, have pronounced bioactivation polymorphism and therefore a flatter distribution than expected from unimodal lognormal distribution.

In Study IV, there is still considerable uncertainty around the risk model, and, ultimately, the calculated excess cancer risk. An example is the simple model used in the present study for relating the formation of DNA-protein crosslinks to cancer risk in mice (22). The single parameter of the model is associated with a SD of at least 78 per cent of the estimated parameter value, according to table 4 in (22). The large uncertainty in this case is caused by the sparseness of the data on extra risk in mice, and would have been handled better by the use of statistical distri-

butions, rather than confidence bounds. The large uncertainty around the parameters of the risk model could easily be incorporated into posterior Monte Carlo simulations. However, the issue of performing and calibrating a toxicokinetic-toxicodynamic link model for risk assessment is a daunting task, and one beyond the scope of the present thesis. Still, the development of link models is a very attractive and sensible approach to an integrated process for risk assessment, and, as in the case of PBPK modeling; Bayesian methods may be of great value in this area. In the future, Bayesian methods may become an essential tool for managing and decreasing parameter uncertainty when faced with complicated, mechanistically based models and sparse data, as suggested by Bois (9).

6. Conclusions

In addition to the considerable information on the population kinetics of methylene chloride, dichloromethane, toluene and styrene gathered in these studies, the results represent a significant contribution to the field of PBPK modeling of risk chemicals, as a number of uncertain elements of PBPK models have been analyzed with a larger statistical rigor than before. The conclusions about PBPK modeling in risk assessment that may be gathered from the thesis work may be summarized:

- The respiratory uptake of volatiles in humans may, at least in some cases, be lower than predicted by reference values on alveolar ventilation. It is thus advisable to check if the reference values really provide reasonable descriptions of the respiratory uptake when PBPK models for inhalation exposure are developed.

- The perfusion of fat tissue may not be described satisfactorily by the use of a single, perfusion-limited fat compartment. Rather, two compartments should be used. Models that incorporate the heterogeneous perfusion of fat tissue may in the future provide more accurate and physiologically realistic descriptions of fat tissue perfusion.

- Suggested reference values seem to provide adequate descriptions of the changing perfusions of various tissues in conjunction with exercise.

- The time course of the change in fat tissue perfusion with exercise is apparently a slow and complex process, which cannot be described accurately using the presently available knowledge from experimental studies.

- Bayesian population modeling is an effective method to separate uncertainty from intra- and inter-individual variability, and may be used to assess other aspects of PBPK modeling than those discussed in the present work.

7. Perspectives

The work presented here only incorporates the available kinetic data on levels of volatile in blood, fat, and exhaled air. There are also extensive concentration data on levels of corresponding metabolites in blood and urine. These data should provide much useful information for assessment of the precision of biomonitoring of occupational exposure. In addition, there are data on many more volatiles, for which the precision of the estimated population risks could be improved significantly by the use of Bayesian methods in a manner similar to that presented here.

There are still a number of areas where more work needs to be done, both experimentally and in modeling, in order to improve the reliability of PBPK models in risk assessment. As mentioned in the Discussion, there is a need for more information on the temporal changes of adipose tissue blood flow and its possible confounding effect on estimated metabolic parameters. The confounding effect of intra-individual variability in alveolar ventilation for volatiles with extensive respiratory elimination also needs to be investigated. These issues need to be tackled experimentally in order to make any firm conclusions.

The assumption of a perfusion-limited behavior in fat tissue is probably not valid. Models that provide physiologically realistic descriptions of the perfusion of fat tissue could be developed using the available references from the physiology literature and validated against the data from previous studies performed at the Institute where the kinetics in fat tissue were monitored.

The scaling factors commonly used in PBPK modeling, also in the present work, have never been subject to rigorous statistical validation.

An important issue in risk assessment is the identification of sensitive subgroups. Here, the possibility of extending the MCMC technique in order to investigate the effect of various covariates, for subsequent use in Monte Carlo estimation of target doses in various subgroups, must be investigated. In addition, more experimental data needs to be collected, as the available data on physiological reference values mostly describe young, Caucasian males. Also, there is a need to incorporate the correlations between parameters into the PBPK-based Monte Carlo simulations used in order to achieve greater reliability of predicted target doses in the general population.

There are research projects regarding the development of more effective MCMC algorithms (80). Hopefully, with the introduction of more effective algorithms and also faster computers, the problem of extensive computation times will be solved in coming years. Hopefully, in a not too distant future, the complicated issues of managing variability and uncertainty in PBPK modeling are universally acknowledged in the field of risk assessment, and the Bayesian population approach is generally accepted as a standard method for PBPK modeling in risk assessment.

8. Summary

Jonsson F. *Physiologically based pharmacokinetic modeling in risk assessment -Development of Bayesian population methods.* Arbete och Hälsa 2001:6.

In risk assessment of risk chemicals, variability in susceptibility in the population is an important aspect. The health hazard of a pollutant is related to the internal exposure to the chemical, *i.e.* the target dose, rather than the external exposure. The target dose may be calculated by physiologically based pharmacokinetic (PBPK) modeling. Furthermore, variability in target dose may be estimated by introducing variability in the physiological, anatomical, and biochemical parameters of the model. Data on these toxicokinetic model parameters may be found in the scientific literature.

Since the early seventies, a large number of experimental inhalation studies of the kinetics of several volatiles in human volunteers have been performed at the National Institute for Working Life in Solna. To this day, only very limited analyses of these extensive data have been performed.

A Bayesian analysis makes it possible to merge *a priori* knowledge from the literature with the information in experimental data. If combined with population PBPK modeling, the Bayesian approach may yield posterior estimates of the toxicokinetic parameters for each subject, as well as for the population. One way of producing these estimates is by so-called Markov-chain Monte Carlo (MCMC) simulation.

The aim of the thesis was to apply the MCMC technique on previously published experimental data. Another objective was to assess the reliability of PBPK models in general by the combination of the extensive data and Bayesian population techniques.

The population kinetics of methyl chloride, dichloromethane, toluene and styrene were assessed. The calibrated model for dichloromethane was used to predict cancer risk in a simulated Swedish population. In some cases, the respiratory uptake of volatiles was found to be lower than predicted from reference values on alveolar ventilation. The perfusion of fat tissue was found to be a complex process that needs special attention in PBPK modeling.

These results provide a significant contribution to the field of PBPK modeling of risk chemicals. Appropriate statistical treatment of uncertainty and variability may increase confidence in model results and ultimately contribute to an improved scientific basis for the estimation of occupational health risks.

Key words: methyl chloride, dichloromethane, toluene, styrene, uncertainty, intra-individual variability, risk assessment, physiologically based modeling, Markov chain Monte Carlo, PBPK, Bayesian, population modeling

9. Summary in Swedish

Jonsson F. *Physiologically based pharmacokinetic modeling in risk assessment -Development of Bayesian population methods.* [*Fysiologiskt baserade modeller inom riskbedömning – Utveckling av Bayesianska populationsmetoder*] Arbete och Hälsa 2001:6.

Vid riskbedömning av kemiska ämnen är en fundamental frågeställning variation i känslighet i befolkningen. Risken som förknippas med exponering för ett viss ämne är relaterad till den inre exponeringen, den s k måldosen, snarare än den yttre exponeringsnivån. Måldoser kan beräknas med hjälp av fysiologiskt baserade farmakokinetiska (PBPK-) modeller. Variabilitet i måldos kan sedan skattas genom att inkorporera variabilitet i modellens fysiologiska, anatomiska och biokemiska parametrar. Uppgifter om dessa toxikokinetiska modellparametrar kan återfinnas i den vetenskapliga litteraturen.

Vid Arbetslivsinstitutet och dess föregångare har sedan nästan 30 år bedrivits studier kring upptag och omsättning av en lång rad lösningsmedelsångor och andra flyktiga ämnen hos frivilliga försökspersoner. Endast mycket begränsade analyser av dessa extensiva data har tidigare utförts.

I en Bayesiansk analys är det möjligt att införliva *a priori*-kunskapen från den vetenskapliga litteraturen med informationen från experimentella data. Om denna metodik kombineras med populationsmodeller så ger ett Bayesianskt angreppssätt skattningar av toxikokokinetiska parametrar för såväl allmänpopulationen som individen. Ett sätt att producera dessa estimat är med hjälp av s k Markovkedje-Monte Carlo- (MCMC-) simulering.

Avhandlingens målsättning var att tillämpa MCMC-metodiken på tidigare publicerade experimentella data. En annan målsättning var att bedöma tillförlitligheten av PBPK-modeller i allmänhet genom kombinationen av extensiva data och Bayesianska metoder.

Populationskinetiken för metylklorid, diklormetan, toluen och styren uppskattades. Den kalibrerade modellen för diklormetan användes för att skatta cancerrisk i en simulerad svensk population vid livslång exponering. Upptaget via andningsvägarna befanns i vissa fall vara lägre än vad som kan antas utifrån litteraturdata. Genomblödningen av fettväv befanns vara en komplex process som kräver speciell uppärksamhet i PBPK-modellstudier.

Dessa resultat utgör ett viktigt bidrag till litteraturen om PBPK-modeller för riskkemikalier. En lämplig statistisk behandling av osäkerhet och variabilitet kan öka pålitligheten hos modellernas förutsägelser och bidrar således till en förbättrad vetenskaplig bas för skattning av hälsorisker i arbetsmiljön.

Nyckelord: metylklorid, diklormetan, toluen, styren, osäkerhet, intra-individuell variabilitet, riskbedömning, fysiologiskt baserade modeller, Markovkedje-Monte Carlo, PBPK, Bayesiansk, populationsmodeller

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Everyone previously and presently employed at the Division of Pharmacokinetics and Drug Therapy.

Susanna Nilsson, my girlfriend ♥♥♥.

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