Arch Toxicol (1996) 70: 347–355

ORIGINAL INVESTIGATION

F.Y. Bois · A. Gelman · J. Jiang · D.R. Maszle · L. Zeise · G. Alexeef

Population toxicokinetics of tetrachloroethylene

Received: 20 April 1995/Accepted: 24 August 1995

Abstract In assessing the distribution and metabolism of toxic compounds in the body, measurements are not always feasible for ethical or technical reasons. Computer modeling offers a reasonable alternative, but the variability and complexity of biological systems pose unique challenges in model building and adjustment. Recent tools from population pharmacokinetics, Bayesian statistical inference, and physiological modeling can be brought together to solve these problems. As an example, we modeled the distribution and metabolism of tetrachloroethylene (PERC) in humans. We derive statistical distributions for the parameters of a physiological model of PERC, on the basis of data from Monster et al. (1979). The model adequately fits both prior physiological information and experimental data. An estimate of the relationship between PERC exposure and fraction metabolized is obtained. Our median population estimate for the fraction of inhaled tetrachloroethylene that is metabolized, at exposure levels exceeding current occupational standards, is 1.5% [95% confidence interval (0.52%, 4.1%)]. At levels approaching ambient inhalation exposure (0.001 ppm), the median estimate of the fraction metabolized is much higher, at 36% [95% confidence interval (15%, 58%)]. This disproportionality should be taken into account when deriving safe exposure limits for tetrachloroethylene and deserves to be verified by further experiments.

Key words Human metabolism · Pharmacokinetics · Population toxicokinetics · Tetrachloroethylene.

F.Y. Bois[1] (✉) · L. Zeise · G. Alexeef
Cal/EPA, Office of Environmental Health Hazard Assessment,
2151 Berkeley Way, Annex 11, Berkeley,
CA 94704, USA

A. Gelman · J. Jiang
Department of Statistics, University of California at Berkeley,
Berkeley, CA 94720, USA

D.R. Maszle
UCB/UCSF Joint Graduate Group in Bioengineering,
University of California at Berkeley, Berkeley,
CA 94720, USA

Present address:
[1]B3E-INSERM U263, Room # 307,
27 Rue Chaligny, F-75012 Paris, France

Introduction

There is currently no general agreement on the value of the fraction of tetrachloroethylene (perchloroethylene, PERC) intake metabolized at low exposure levels in humans. Still, this number is of importance in determining the health risks posed by exposure to PERC: PERC is carcinogenic in animals via one or several of its metabolites (Odum et al. 1988; Green et al. 1990; Alexeeff et al. 1992); therefore the fraction metabolized is likely to be a better measure of toxic exposure than PERC exposure itself. For humans this fraction is difficult to measure directly but can be estimated with a physiological toxicokinetic model (Gerlowski and Jain 1983; Balant and Gex-Fabry 1990; Bois et al. 1990; Andersen et al. 1993). Such models (Fig. 1) allow the simulation of a variety of end-points (e.g. metabolite concentrations) in specific organs, while providing the opportunity to use relevant prior information (usually published literature) on physiological parameters, such as blood flows, organ volumes etc. Yet, some parameters, typically those controlling metabolism, are not known with precision. Proper statistical inference regarding the value of these parameters is therefore necessary.

In addition, our interest lies in inference about PERC metabolism in humans, i.e. in a diverse population, rather than in any one individual studied in published experiments. We therefore designed a statistical model describing the relationships between individual and population physiological parameters to estimate population variability (Sheiner 1984; Racine-Poon and

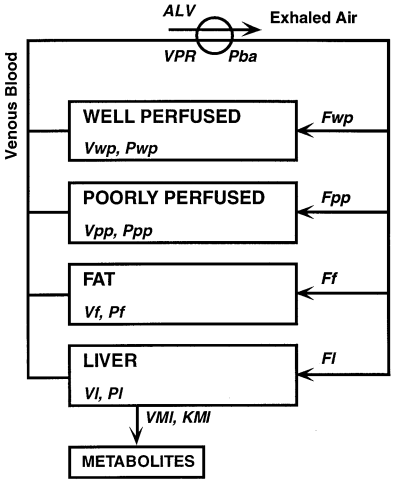

Fig. 1 Schematic representation of the 4 compartment physiological model used to simulate the distribution and metabolism of PERC. The symbols are: V, volumes; F, blood flows; P, partition coefficients; Vml, and Kml, Michaelis-Menten coefficients; ALV, alveolar ventilation rate; VPR, ventilation over perfusion ratio

Table 1 Measured individual characteristics of the six human male volunteers exposed to PERC by Monster et al. (personal communication; 1979)

Subject	Body mass (kg)	Lean body mass (kg)	Minute volume at rest (l/min)	Age (years)
A	70	62	7.6	31
B	82	71	11.6	22
C	82	71	10.0	21
D	86	74	11.3	28
E	67	61	12.3	27
F	77	61	8.8	25
Mean	77[a]	66[a]	10[a]	26[b]

[a] Geometric mean
[b] Arithmetic mean

Smith 1990; Sheiner and Ludden 1992; Smith and Wakefield 1994; Wakefield et al. 1994). Linking a population model to a physiological toxicokinetic model has already been achieved by Droz et al. (1989a, b), but fitting the linked models has never been done, to our knowledge, and may seem like wishful thinking with regard to modeling and computation. In fact, up to now, little consideration has generally been given to statistical issues when using physiological models: the task seems daunting, given the number of parameters involved in these models and the relative paucity of relevant data. Yet, for such difficult problems, the use of Bayesian numerical methods is promising (Woodruff et al. 1992; Woodruff and Bois 1993). Bayesian statistics also provide a natural way to merge a priori knowledge, gained by implementing a physiological model, with the in vivo experimental data. We describe the application to our model of Markov chain Monte Carlo simulation, which is a particularly simple and powerful tool. A similar approach to pharmacokinetic modeling is presented by Wakefield et al. (1994) and Smith and Wakefield (1994), for classical one-compartment models. By using a physiological model, we increase the number of parameters but can take advantage of a large body of prior information on the parameter values. We report predictions and confidence bounds on the fraction of PERC metabolized at low dose in humans. We discuss how the method can improve our use of toxicokinetic modeling for exposure to toxic substances in the air.

Materials and methods

Data and models

The data comprised the concentrations of PERC in exhaled air and venous blood for six male volunteers exposed to 72 ppm PERC in an inhalation chamber during 4 h (Monster, personal communication; Monster et al. 1979). PERC concentrations were measured over the week following exposure. Two exposure levels were used: 72 ppm (204 μg/l) and 144 ppm (409 μg/l). In addition, a set of physiologic measurements was obtained on each individual (Table 1).

We used a physiological model in which the human body is divided into four compartments: poorly perfused tissues, well perfused tissues, fat, and liver (Bois et al. 1990) (Fig. 1). Compartments are assumed to be homogeneous and distribution limited by blood flow. Pulmonary exchanges are modeled by assuming instantaneous equilibrium between alveolar air, venous blood and arterial blood. Differential equations of the form $\partial C_i/\partial t = (C_{art} - C_i/P_i)F_i/V_i$ describe the time dependence of the concentration C_i of PERC in each compartment i as a function of blood flow F_i, volume V_i, arterial blood concentration C_{art} and partition coefficient P_i. These equations are linear, except for the liver compartment in which a Michaelis-Menten term describes the metabolic clearance of PERC. For this compartment,

$$\frac{\partial C_l}{\partial t} = \frac{F_l}{V_l}\left(C_{art} - \frac{C_l}{P_l}\right) - \frac{V_{\max} C_l}{K_m + C_l V_l}$$

where $V_{\max}$ is the maximum rate of metabolism and K_m the Michaelis constant. The differentials were solved using our own software, MCSim (available from the first author). This model allows us to compute, for given parameter values and exposure conditions, various quantities relevant for our purpose: concentration of PERC in blood or exhaled air, and quantity of PERC metabolized in a given period of time.

The statistical model was constructed using a hierachical population approach, as described in Fig. 2. It has two major components: the individual level and the population level. At the individual's level, for each of six subjects, exhaled air and blood concentrations ($\mathbf{y}$) were measured experimentally. The expected values of the exhaled air and blood concentrations are a function (f) of exposure level ($\mathbf{E}$), time ($\mathbf{t}$), a set of physiological parameters of unknown values ($\boldsymbol{\theta}$), and a set of measured, covariate parameters ($\boldsymbol{\varphi}$). $\mathbf{E}$, $\mathbf{t}$, $\boldsymbol{\theta}$, and $\boldsymbol{\varphi}$ are subject-specific. The function f is the nonlinear physiological model, described above. The concentrations actually observed in expired air and blood are also affected by measurement errors, which are assumed, as usual, to be independent and log-normally distributed, with a mean of zero and a variance $\boldsymbol{\sigma}^2$ (on the log scale). The variance vector $\boldsymbol{\sigma}^2$ has two components, $\boldsymbol{\sigma}_1^2$ for the measurements in blood, and $\boldsymbol{\sigma}_2^2$ for the measurements in exhaled air, because these measurements have different experimental protocols and are therefore likely to have different precisions.

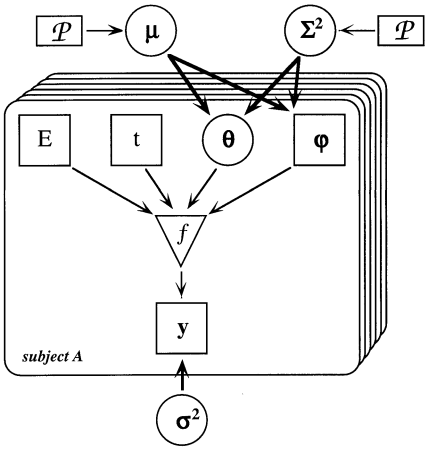

Fig. 2 Graph of the statistical model describing the dependence relationships between several groups of variables. Symbols are: $\boldsymbol{\mu}$ mean population parameters; $\boldsymbol{\Sigma}^2$ variances of the parameters in the population; **E** PERC exposure concentrations; **t** experimental sampling times; $\boldsymbol{\theta}$ unknown physiological parameters; $\boldsymbol{\varphi}$ measured physiological parameters; *f* toxicokinetic model; **y** measured PERC concentrations in blood or exhaled air; $\boldsymbol{\sigma}^2$ variance of the experimental measurements

Three types of nodes are featured in Fig. 2:

- Square nodes represent variables for which the values are known by observation, such as **y** or $\boldsymbol{\varphi}$, were fixed by the experimenters; for example **E** and **t**; or were fixed by ourselves, for example the prior on $\boldsymbol{\mu}$ and $\boldsymbol{\Sigma}^2$.
- Circle nodes represent unknown variables, such as $\boldsymbol{\theta}$, $\boldsymbol{\sigma}^2$, $\boldsymbol{\mu}$, or $\boldsymbol{\Sigma}^2$.
- Following the notation of Thomas et al. (1992), the triangle represents the deterministic physiological model *f*.

An arrow between two nodes indicates a direct statistical dependence between the variables of those nodes.

A priori parameter distributions

To take into account known physiological dependencies between the toxicokinetic model parameters (e.g., between organ volumes and body weight, or alveolar ventilation rate and cardiac output), several of them were linked to the lean body mass or other parameter values, via scaling functions (Adolph 1949; Davidson et al. 1986; Mordenti 1986; Ings, 1990) (Table 2). In brief, volumes are input as fractions of the lean body weight, flows as fractions of cardiac output (itself obtained by multiplying the minute volume, which is the total pulmonary ventilation flow, by the ventilation over perfusion ratio), and the maximum rate of metabolism as

Table 2 Prior parameters, truncations, and summary of the posterior (fitted) distributions for the population means and standard deviations of the scaling coefficients of the PERC model parameters in humans [a,b,c]. The posterior distributions are established using the last 5000 iterations of the five runs performed

Scaled parameter		Prior on μ		Prior on Σ		Posterior
	Multiplier	exp (M)	exp (S)	Truncation[d]	exp (Σ_0)	exp(μ) × ÷ exp(Σ)
Ventilation over perfusion ratio (*VPR*)	1	1.6	1.3	3	1.3	1.19 × ÷ 1.13
Blood flows						
Well perfused tissues (*Fwp*)	$0.7 \times MV/VPR$	0.48[e]	1.2[f]	3	1.2	0.637 × ÷ 1.06
Poorly perfused tissues (*Fpp*)	$0.7 \times MV/VPR$	0.20	1.2[f]	3	1.2	0.129 × ÷ 1.11
Fat (*Ff*)	$0.7 \times MV/VPR$	0.07	1.2[f]	3	1.2	0.0488 × ÷ 1.12
Liver (*Fl*)	$0.7 \times MV/VPR$	0.25	1.1[f]	3	1.1	0.179 × ÷ 1.11
Volumes						
Well perfused tissues (*Vwp*)	*LBM*	0.28[e]	1.2[f]	3	1.2	0.196 × ÷ 1.09
Poorly perfused tissues (*Vpp*)	*LBM*	0.56[e]	1.2[f]	3	1.2	0.641 × ÷ 1.03
Liver (*Vl*)	*LBM*	0.033	1.1[f]	3	1.1	0.033 × ÷ 1.04
Blood/air partition coefficient (*Pba*)	1	12	1.5	3	1.3	16.0 × ÷ 1.11
Tissue/blood partition coefficients					1.3	
Well perfused tissues (*Pwp*)	1	4.8	1.5	3	1.3	1.92 × ÷ 1.12
Poorly perfused tissues (*Ppp*)	1	1.6	1.5	3	1.3	2.9 × ÷ 1.15
Fat (*Pf*)	1	125	1.5	3	1.3	84.1 × ÷ 1.28
Liver (*Pl*)	1	4.8	1.5	3	1.3	3.08 × ÷ 1.12
Max. rate of metabolism in liver (*VMl*)	$LBM^{0.7}$	0.042	10	2	2	0.00191 × ÷ 1.45
Km in liver (*KMl*)	1	16	10	2	1.5	0.729 × ÷ 1.20

[a] Scaled parameter = multiplier × scaling coefficient (a multiplier of 1 implies that no scaling is made). Units: weights in kg, flows in l/min, volumes in l, *VMl* in mg/min, *KMl* in mg

[b] Body mass (*BM*), lean body mass (*LBM*), minute volume (*MV*), volume of fat (*Vf*) are explicitly given in Monster et al. (1979) and summarized in Table 2

[c] For all parameters the scaling coefficients are assumed to be a priori lognormally distributed

[d] Truncation is expressed in terms of *n*, the number of SD added of subtracted to the mean. In natural space the bounds were therefore exp(M ± *n*S)

[e] For these parameters the reparametrization lead to actual means of 0.47, 0.27, and 0.55, respectively (see text)

[f] For these parameters a reparametrization was used with actual SDs at 1.17, 1.22, 1.27, 1.15, 1.36, and 1.17, respectively (see text)

a power function of lean body weight. The other parameters were unscaled. The scaling coefficients were the actual parameters used in input.

At the population level, we assumed that the each component of the $\boldsymbol{\theta}$ parameter set was distributed log-normally, with population averages $\boldsymbol{\mu}$ and variances $\boldsymbol{\Sigma}^2$ (in log scale). We have some a priori knowledge of $\boldsymbol{\mu}$ and $\boldsymbol{\Sigma}^2$, at least in the form of standard values (International Commission on Radiological Protection (ICRP), 1975). Information about the distribution of an individual's $\boldsymbol{\theta}$ parameter values is given by the experimental data and by the population parameters. We assigned a priori truncated normal distributions to the population means $\boldsymbol{\mu}$ (with parameters **M** and **S**) and inverse gamma distributions for the population variances $\boldsymbol{\Sigma}^2$. We defined prior value for the hyper-parameters **M**, **S**, and $\boldsymbol{\Sigma}^2$, on the basis of the literature (Guberan and Fernandez 1974; International Commission on Radiological Protection (ICRP) 1975; Åstrand 1983; Fiserova-Bergerova 1983; Ward et al. 1988; Koizumi 1989; Williams and Leggett 1989; Bois et al. 1990). The choice of values for these parameters and the bounds for truncation (expressed as a number of standard deviations to be subtracted or added to the mean) are summarized in Table 2. In setting uncertainties, we tried to be conservative and set the prior variances higher rather than lower when there was ambiguity in the biological literature (for example, with the partition coefficients). For convenience, we give in Table 2 the value of exp(**M**), i.e. the geometric mean, exp(**S**) and exp($\boldsymbol{\Sigma}_o$), which lie on the natural scale.

The values used for organ masses, when expressed as fractions of lean body weight, are usually considered as reference values for 35-year-old males (International Commission on Radiological Protection (ICRP) 1975; Williams and Leggett 1989). Volumes in liters and masses in kilograms have the same values, since a density of 1.0 is assumed for all tissues, except for the fat (density 0.92). Both the uncertainty on μ and the heterogeneity of the fraction volumes in the population are estimated to be of the order of 10–20% (coefficient of variation), depending on the tissue group. Truncation was set to ± 3 standard deviations around M.

The geometric means of the fractions of cardiac output going to different compartments were set to usually accepted reference values (International Commission on Radiological Protection (ICRP) 1975; Williams and Leggett 1989). The mean ventilation over perfusion ratio, VPR, was set at 1.6 (Åstrand, 1983), since the subjects were allowed some activity after exposure. Exp(S) and exp(Σ_o) were set at 1.1 for the liver blood flow, 1.2 for the flows to other tissues, and 1.3 for VPR. This corresponds approximately to 10–30% variability. Truncation was set to ± 3 standard deviations.

The values found in the literature for PERC blood/air partition coefficient range from 9.1 to 18.9 (Guberan and Fernandez 1974; Fiserova-Bergerova 1983; Ward et al. 1988; Koizumi 1989), with a geometric mean of 12, which we adopt here. The geometric means of the tissue over blood partition coefficients were set to 4.8 for the liver and well perfused tissue, 1.6 for the poorly perfused tissue, and 125 for the fat. These correspond to the geometric means of the values published independently (for the rat) by Koizumi (1989) and Ward et al. (1988). While PERC partition coefficients do not vary with hematocrit (Morgan et al. 1970), they could still vary within an individual (depending on fasting, for example), by a factor of 2 (Fiserova-Bergerova 1983). Therefore exp(S) was set at 1.5 and truncation was set at ± 3 standard deviations for all partition coefficients. This truncation corresponds to bounds 3.56 and 40.5 for the blood/air partition coefficient. Exp(Σ_o) was set to 1.3.

Some prior estimates for the population's maximum rate of metabolism, VMl, and for the Michaelis-Menten coefficient, KMl, were obtained when fitting the model to animal data (Bois et al. 1990). For VMl, a value of 1.2 mg/min is obtained when extrapolating the value of 0.006 mg/min found in mice. A value of 0.3 mg/min is obtained when extrapolating the value of 0.008 mg/min found in rats. Extrapolation was performed by allometric scaling using body weight to the power 0.7 (Mordenti 1986). Independently, data from in vitro experiments (Reitz 1992) indicate that VMl value (in mg/min per kg) in humans is approximately one-eighth of that for the mouse and two-fifth of the rat value. This translates for humans into values of 1.4 mg/min and 0.64 mg/min from mouse and rat data, respectively. We adopt for humans a geometric mean of 0.7 mg/min, bracketed by the extrapolated animal values. A large uncertainty is still associated with this number. The animal values are themselves uncertain (Bois et al. 1990) and the agreement of the two extrapolation methods could be fortuitous. We choose a value of 10 for exp(S), and truncation at $\pm 2S$, in log space. This truncation corresponds to ± 2 orders of magnitude around the geometric mean. Since in vitro human data (Reitz 1992) indicate a population coefficient of variation of approximately 2, we set exp(Σ_o) at 2. Thus, we believe these parameters to vary in Monster et al.'s subjects by about a factor of 2, but we are uncertain by a factor of 10 as to their population mean. It would be difficult to express this sort of uncertainty without an explicit hierarchical model.

For the Michaelis-Menten coefficient we found a value of 12 mg/l for mice and 6.5 mg/l for rats (Bois et al. 1990). We adopt a geometric mean of 9 mg/l, assuming that this parameter does not change appreciably across species. The model parameter KMl is in units of quantity rather than concentration and was set to 16 (9 times the average liver volume for a lean body weight of 55 kg). A large uncertainty is still associated with this number and we set exp(S) to 10, and truncation at ± 2S, that is ± 2 orders of magnitude. We assume a population coefficient of variation of approximately 1.5 and thus set exp(Σ_o) at 1.5.

At the individual level we had no prior information for most of the parameters (except for those in Table 1), so their prior distributions were entirely determined by the population parameters, μ and Σ^2, and by the data.

As a consequence of scaling, some of the parameters are constrained by definition: for each individual k, the fractions of blood flow to each compartment have to sum to 1. Also, the scaling coefficients of the organ volumes have to sum to 0.873 (the fraction of lean body weight not including bones), for each individual. These constraints make Monte Carlo sampling difficult and it is preferable to remove them. We reparametrized the model in terms of a new set $\boldsymbol{\psi}$ of parameters which automatically satisfy the sum constraints (Gelman 1995). The normal models with μ_l and Σ_l were then applied to the new parameters.

Statistical computations

A Bayesian analysis allowed us to combine two forms of information: "prior knowledge" from the scientific literature, and "data" from Monster's experiments, in the context of the physiological compartmental model. Neither source of information is complete. If prior knowledge were sufficient, the experiments would not have had to be done, but Monster's data alone are insufficient to pin down the parameters to reasonable values. Our goal was to fit the data using scientifically plausible parameter values.

The second interesting feature of the Bayesian approach is that it produces a posterior distribution for the parameters, rather than a mere point estimate. Thus, the analysis outputs distributions of parameter values that are consistent with both the data and the prior information. Our statistical analysis yields distributional estimates (posterior distributions) of the parameters for each subject and for the population.

Current standard practice in Bayesian statistics is to summarize a complicated high-dimensional posterior distribution by random draws of the vector of parameters, in this case, from the distribution $P(\boldsymbol{\theta}, \boldsymbol{\mu}, \boldsymbol{\Sigma}^2, \boldsymbol{\sigma}^2 | \mathbf{M}, \mathbf{S}, \text{data})$. The simulations can then be used to compute posterior distributions of estimands of interest, including individual parameters, and also derived quantities such as the proportion of PERC metabolized under specified conditions. Because $\boldsymbol{\theta}$ has many components, we use a combination of Gibbs sampling and Metropolis-Hasting sampling to perform a random walk through the posterior distribution. These samplings are iterative procedures, particularly convenient in the case of hierarchical

models. They belong to a class of Markov chain Monte Carlo techniques which has recently received much interest (Gelfand et al. 1990; Gelfand and Smith 1990; Gelfand et al. 1992; Smith 1991; Tanner 1991; Gelman, 1992; Wakefield et al. 1994). The sampling distributions of the different components of the model are given in the Appendix. Five independent Monte Carlo runs were performed. Convergence was monitored using the method of Gelman and Rubin (1992). Details of the technique are described in a technical report available from Dr. Bois.

To obtain the distribution of the fraction of PERC metabolized at low and high exposure by each of the six subjects, two scenarios were simulated, using as input the parameter values generated in last 5000 iterations of each run. Continuous exposures to PERC (0.001 ppm and 50 ppm) were simulated over 3 weeks. The amount metabolized the last day was recorded and divided by the amount inhaled on the same day. The amount inhaled is equal to the alveolar ventilation volume for a day times the PERC inhalation level. Similar simulations were performed for the population by sampling one random parameter vector from $N(\boldsymbol{\mu}, \boldsymbol{\Sigma})$ for each of the 25 000 estimates of $\boldsymbol{\mu}$ and $\boldsymbol{\Sigma}$. This accounts for parameter covariance, since the 5×5000 individual and population parameter sets are random draws from their joint (multivariate) distributions, not just from the marginal distributions. For these simulations lean body mass, mass of fat as fraction of the lean mass, and minute volume were also sampled lognormally, with geometric means equal to that of the six subjects of Monster et al. Standard deviations for these parameters were set at log(1.3), log(1.2) and log(1.2), respectively.

Results and discussion

Model fit

Defining prior distributions for the physiological parameters was difficult. While it is well known that these parameters exhibit a wide range of interindividual variability, the only values readily available, and those always used in physiological modeling, are "reference" values for young Caucasian males. Such reference values artificially reduce the population variance estimates. What is really needed is a database giving access to the population distributions of important physiological parameter values. Such a database would be usable for all types of physiological modeling, and for both toxicants and drugs. Due to the current lack of information, we had to use "reference" values to the population means, and gave reasonable guesses for population standard deviations and truncation limits. We also had to choose the shape of prior, and selected the lognormal distribution, which is often used for physiological parameters. The posterior shape, however, is free to be different, and can take any form. Further, we have recently observed, in another application (unpublished), that the shape of the prior distributions has little impact on the final results: the data actually "dominate" the prior.

The use of the Markov chain simulations, which reached approximate convergence in about 10 000 iterations, has allowed us to obtain an excellent fit to the data of Monster et al., while maintaining scientifically plausible parameter values. Further simulations did not affect the results appreciably. Figure 3 shows the

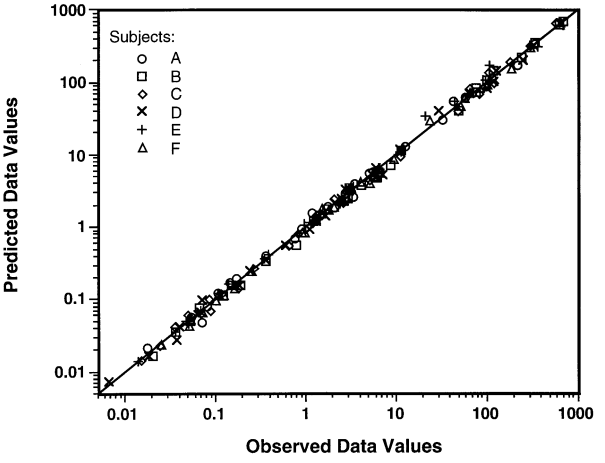

Fig. 3 Predicted versus observed data values (blood and exhaled air concentrations) for the last iteration of the first run

data values predicted for each individual versus their observed counterparts (all data values are concentrations). Predictions were made with the parameter values of the last iteration of the first run. This iteration is not "better" than any of the last 5000; it is just representative of the set. For an optimal fit, all points would fall on the diagonal. Such an adjustment is not expected given the analytical measurement errors in the data, but the deviations are small and the fit seems reasonable compared to other models fit to these and similar data (Hattis et al. 1990; Bois et al. 1991).

To check the model, we simulated another inhalation experiment on human volunteers. Opdam and Smolders (1986) exposed six subjects to constant levels of PERC ranging from 0.5 to 9 ppm, and followed alveolar concentration during exposure (up to 50 min). Simulations were performed using the 25 000 posterior estimates of $\boldsymbol{\mu}$ and $\boldsymbol{\Sigma}$ (data not shown). The model adjustment is good overall (i.e. all data points are included in the 95th centile envelope), even though exposure levels were 5–100 times lower than those used in the studies of Monster et al.

Posterior distributions — fraction of PERC metabolized

Among the results are presented the posterior distributions of all parameter values for individuals (whose precision is affected by measurement errors) and for the population (whose precision depends on population heterogeneity). The last column of Table 2 summarizes the distributions of the population parameter values obtained in the last 5000 iterations of the five runs performed (results of the five runs are pooled, and the distributions are established with 25 000 values). The location of many parameters is noticeably different from the corresponding prior mean. Yet, the posterior distributions for the parameters are consistent with

their prior distributions (i.e. within 1 or 2 prior SDs), indicating that the good fit to the data was not obtained by "overfitting". In particular, values of the scaling coefficient of metabolic parameters, which are crucial for the determination of the fraction metabolized, are quite well identified (individual standard deviation corresponding approximately to a factor of 1.5). The mean of the scaling coefficient of the maximum rate of metabolism is 20 times lower than our prior estimate, which was imprecise. This implies that the maximum rate of PERC metabolism in humans is much lower than the values extrapolated from rodents on the basis of body weight to the power 0.7. Inter-individual variations of a factor of 2 are not uncommon for metabolic parameters (and such a range was even found among the six subjects). Similar variability for classical pharmacokinetic parameters of PERC (clearance, volume of distribution, etc.) was found in a small group of subjects very similar to those studied here (Opdam 1989). Wider variations would certainly be found when observing a larger population.

Joint distributions of the fraction of PERC metabolized by the six subjects at high and low exposures can be computed by the model (Fig. 4). Marginal distributions can indeed also be obtained from these results. The population distributions of the fraction metabolized are quite spread. At low exposure (Fig. 5) the mean, and standard deviation of 25 000 draws of the fraction metabolized in the population are 36% and 11%, respectively. At high exposure these numbers are 1.7% and 0.95%, respectively. Confidence bounds can be obtained as percentiles of these distributions. At low exposure the 95% confidence interval of the fraction metabolized is (15%, 58%); at high exposure it is (0.52%, 4.1%). This high exposure estimate is in agreement with the figures of Monster et al. (1979) for the recovery of inhaled PERC: in the experiments unchanged PERC recovery was 80–100%, and approximately 2% of the inhaled dose was recovered in urine as trichloroacetic acid. However, risk assessments using a fraction metabolized calculated directly from the experiment, without considering exposure concentration, would be likely to underestimate the potential risks at low PERC exposure, by a factor of approximately 20.

The relationship between fraction of PERC metabolized in 1 day, after 3 weeks continuous inhalation exposure, and exposure level is presented on Fig. 6. At low exposure levels the fraction metabolized remains constant, since metabolism is linear. Saturation starts occurring above 1 ppm and is almost complete at 10 ppm. At higher levels the fraction metabolized decreases linearly with exposure since the quantity metabolized per unit time is at its maximum.

A recent report examines the impact of variability in some of the parameters of a physiological model of PERC on predictions of metabolite dose (Gearhart et al. 1993). Animal parameter distributions were obtained from the literature or in vitro experiments for

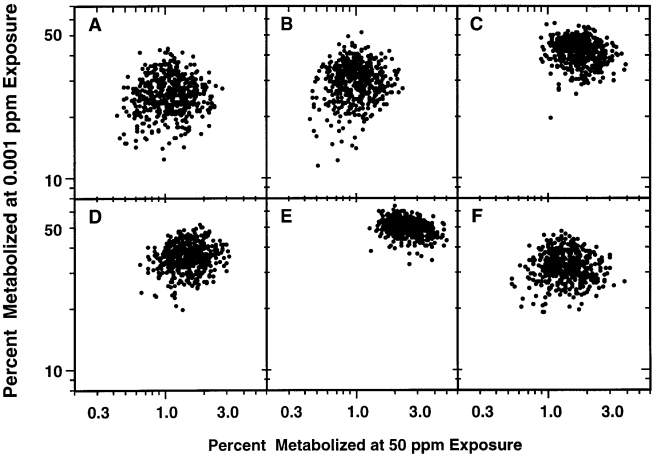

Fig. 4 Estimates of the fraction of PERC metabolized per day for a continuous inhalation exposure to 50 ppm versus estimated fraction metabolized at 0.001 ppm, for the subjects of Monster et al. experiments. Only one point in 50, out of a total of 25 000, is presented

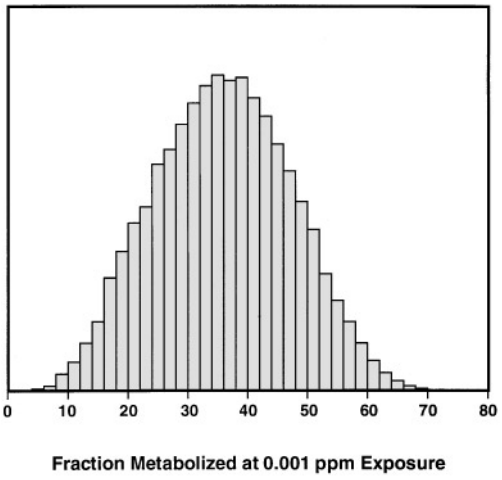

Fig. 5 Estimated population distribution of the fraction of PERC metabolized per day for a continuous inhalation exposure to 0.001 ppm PERC in the air

some of the model parameters (flows, volumes, and partition coefficients). These distributions incorporate a mix of uncertainty (measurement errors) and variability (results from several animals were pooled). The metabolic parameters were visually fitted. Human parameter values or distributions were obtained from one individual (partition coefficients), from the literature (flows and volumes) or by extrapolation from animals (metabolic parameters). The authors conclude that "parameter uncertainty is not a significant potential source of variability in the use of PBPK models in risk assessment". Such a conclusion suffers from confusion between uncertainty and variability, and from the limited scope of the study on which it is based. It is true that flows or volumes alone may not have a large

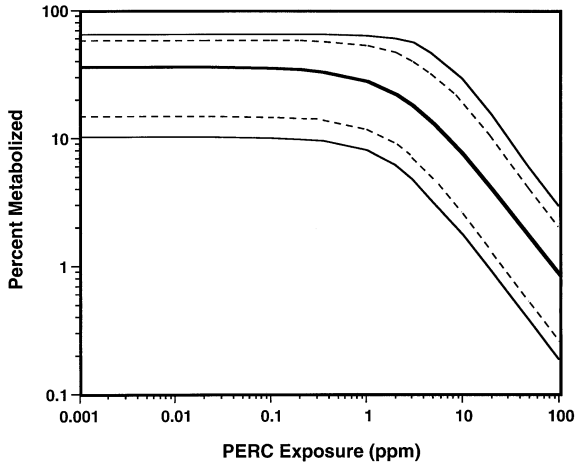

Fig. 6 Relationship between fraction of PERC metabolized and inhalation exposure level. The *thick line* corresponds to the mean population model predictions. The *thin lines* bracket the 99% confidence interval and the *dotted lines* the 95% confidence interval, over 25 000 simulations

impact on the amount of metabolites produced at continuous low exposure. However, they are much more influential in the short term experiments typically available for humans or animals, and will significantly affect the estimation uncertainty for the metabolic parameters. This effect is not observable when estimation is decoupled (i.e. when one or two parameters are independently fitted), but is manifest in this study: at low exposure many parameters do condition the amount metabolized, even if indirectly.

Hattis et al. (1990) reviewed the literature on model-based estimates of the fraction of PERC metabolized at low dose (1 ppm). Previous estimates range from 2 to 86%. None of these were obtained by a complete statistical estimation procedure. The lowest estimates, not surprisingly, were obtained from models which assumed linear metabolism and were parametrized with high exposure data. Note that even though our model contains a nonlinear Michaelis-Menten term for metabolism it was not constrained to behave nonlinearly. Had metabolism actually been linear in Monster et al.'s volunteers, the estimate of KMl would have been driven to the upper bound of its prior distribution, where the Michaelis-Menten term would behave linearly. This did not happen and KMl stabilized around a value 2000 times smaller than its a priori upper bound (but the prior SD, on the log scale, corresponded to an order of magnitude). Although the exposure levels were high in the experiments (72 and 144 ppm), the time course of PERC concentrations in blood and exhaled air was followed with sufficient precision over a extended period of time, and over a large range of tissue concentrations. This experimental design is sufficiently powerful to allow a reasonable identification of VMl and KMl values, albeit with some covariance.

It is important to point out that large variations also exist between individuals—a factor two difference is seen between similar subjects A and E (in Fig. 4). Variations of the fraction metabolized are even larger in the simulated general population (Fig. 5) where a factor of 30 difference (at low exposure) is observed between the highest and lowest estimates among 25 000. We did not explicitly model intraindividual variability because of limitations in the data. Each subject was exposed twice, but to different concentrations of PERC. Note, however, that for a given subject the same set of parameter values gives a very good fit to all data, despite the fact that they were obtained at different periods. It is therefore likely that intraindividual variability had little impact in this study. Future experiments should try to include repeated exposures to confirm this result. They should also be performed at lower exposure levels to confirm experimentally our present findings. While uncertainty could be reduced by additional analyses, population variability, which in this study is approximately as large as uncertainty about individual subjects, could increase when more subjects are included.

These results are indeed conditioned by the use of a particular dataset. We did not develop new data because it is unethical to unnecessarily expose volunteers to toxic chemicals. Before doing so, we preferred to reanalyze previously collected high quality data with improved tools. The method presented here is of general applicability. Coupling Bayesian statistical estimation to toxicokinetic models takes full advantage of these two powerful tools.

Acknowledgements This work was supported by the Office of Environmental and Health Hazard Assessment, California Environmental Protection Agency and in part by contract number 91–12835 of the American Lung Association of California. A.G. is also supported by NSF grants DMS-9404305 and DMS-9457824. We thank Dr. A. Monster for sharing his data and for his comments.

Appendix

The conditional posterior distribution of $\boldsymbol{\sigma}^2$ (sampled at each step of the sampler) is, for $m = 1$ or 2 (either blood or exhaled air):

$$\sigma_m^2 | \text{all other parameters} \sim \text{Inverse} - \chi^2\left(L, L^{-1} \sum_i \sum_j [\log(y_{ijm}) - \log(f_m(\boldsymbol{\Psi}, \boldsymbol{\varphi}, E_j, t_i))]^2\right) \quad (1)$$

where L is the total number of observations of type m for all measurements on all six subjects. For a given individual, i indexes the measurement time, and j the dose (the other symbols have been described in the main text). We draw samples from the inverse-gamma distribution using Cheng's rejection algorithm GB (Devroye 1986, Section IX.3).

The conditional posterior density for any component of $\boldsymbol{\Psi}$, ψ_{kl}, is:

$$P(\psi_{kl} | \mathbf{y}, \text{all other } \psi\text{'s}, \boldsymbol{\varphi}, \boldsymbol{\sigma}^2, \mathbf{E}, \mathbf{t}, \boldsymbol{\mu}, \boldsymbol{\Sigma}^2) \propto \exp\left(-\frac{1}{2} \Sigma_l^{-2} [\psi_{kl} - \mu_l]^2\right) \cdot \prod_i \prod_j \prod_m$$

$$\times \exp\left(-\frac{1}{2}\sigma_m^{-2}\left[\log(y_{ijm}) - \log(f_m(\boldsymbol{\Psi}, \boldsymbol{\varphi}, E_j, t_i))\right]^2\right) \quad (2)$$

where $k = 1, \ldots, 6$ (6 subjects), and $l = 1, \ldots, 18$ (18 model parameters). Because of f (the nonlinear pharmacokinetic model) this cannot be written in closed form as a function of $\boldsymbol{\Psi}$. Instead of directly sampling ψ_{kl} from this conditional distribution, we sample a "proposal" value from $\mathcal{N}(\psi_{kl}, (S_{kl}/20)^2)$, that is centered at the current value of ψ_{kl}, and with a constant standard deviation proportional to S_{kl}. The proportionality factor was set to 20 after preliminary runs. We then either update the value of ψ_{kl} to that new value, or leave it unchanged, based on a Metropolis acceptance/rejection rule (Gelfand and smith 1990; Gelman 1992).

The conditional distributions of the population parameters μ_l and ${\Sigma_l}^2$ are normal. For each l:

$$\mu_l|\mathbf{y}, \text{ all other parameters} \sim \mathcal{N}\left(\frac{M_l\Sigma_l^2 + S_l^2\sum_k \psi_{kl}}{nS_l^2 + \Sigma_l^2}, \frac{\Sigma_l^2 S_l^2}{nS_l^2 + \Sigma_l^2}\right) \quad (3)$$

$$\Sigma_l^2|\mathbf{y}, \text{ all other parameters} \sim \text{Inverse}$$

$$-\chi^2\left(n + v, \frac{1}{n+v}\left[v\Sigma_{0l}^2 + \sum_k (\psi_{kl} - \mu_l)^2\right]\right) \quad (4)$$

using the notation of Gelman (1995), and remembering that $n = 6$.

References

Adolph EF (1949) Quantitative relations in the physiological constitution of mammals. Science 109: 579–585

Alexeeff GV, Lewis DC, Zeise L, Cox DN, Schunk HH (1992) Health effects of perchloroethylene. California Department of Health Services, Office of Environmental Health Hazard Assessment, CAL/EPA

Andersen ME, Krewski D, Withey JR (1993) Physiological pharmacokinetics and cancer risk assessment. Cancer Lett 69: 1–14

Åstrand I (1983) Effect of physical exercise on uptake, distribution and elimination of vapors in man. In: Fiserova-Bergerova F (ed) Modeling of inhalation exposure to vapors: uptake, distribution, and elimination. CRC Press, Boca Raton, Florida, pp 107–130

Balant LP, Gex-Fabry M (1990) Physiological pharmacokinetic modelling. Xenobiotica 20: 1241–1257

Bois FY, Zeise L, Tozer TN (1990) Precision and sensitivity analysis of pharmacokinetic models for cancer risk assessment: tetrachloroethylene in mice, rats and humans. Toxicol Appl Pharmacol 102: 300–315

Bois FY, Woodruff TJ, Spear RC (1991) Comparison of three physiologically-based pharmacokinetic models of benzene disposition. Toxicol Appl Pharmacol 110: 79–88

Davidson IWF, Parker JC, Beliles RP (1986) Biological basis for extrapolation across mammalian species. Regul Toxicol Pharmacol 6: 211–237

Devroye L (1986) Non-uniform random variate generation. Springer, New York

Droz PO, Wu MM, Cumberland WG (1989a) Variability in biological monitoring of organic solvent exposure—II. Application of a population physiological model. Br J Ind Med 46: 547–558

Droz PO, Wu MM, Cumberland WG, Berode M (1989b) Variability in biological monitoring of solvent exposure—1. Development and validation of a population model. Br J Ind Med 46: 447–460

Fiserova-Bergerova V (1983) Gases and their solubility: a review of fundamentals. In: Fiserova-Bergerova F (ed) Modeling of inhalation exposure to vapors: uptake, distribution, and elimination. CRC Press, Boca Raton, Florida, pp 3–28

Gearhart JM, Mahle DA, Greene RJ, Seckel CS, Flemming CD, Fisher JW, Clewell HJ III (1993) Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). Toxicol Lett 68: 131–144

Gelfand AE, Smith AFM (1990) Sampling-based approaches to calculating marginal densities. J Am Statist Assoc 85: 398–409

Gelfand AE, Hills SE, Racine-Poon A, Smith AFM (1990) Illustration of Bayesian inference in normal data models using Gibbs sampling. J Am Statist Assoc 85: 972–985

Gelfand AE, Smith AFM, Lee T-M (1992) Bayesian analysis of constrained parameter and truncated data problems using Gibbs sampling. J Am Statist Assoc 87: 523–532

Gelman A (1992) Iterative and non-iterative simulation algorithms. Comput Sci Stat 24: 433–438

Gelman A (1995) Method of moments using Monte Carlo simulation. J Comput Graphic Stat (in press)

Gelman A, Rubin DB (1992) Inference from iterative simulation using multiple sequences (with discussion). Stat Sci 7: 457–511

Gerlowski LE, Jain RK (1983) Physiologically based pharmacokinetic modeling: principles and applications. J Pharm Sci 72: 1103–1127

Green T, Odum J, Nash JA, J.R. F (1990) Perchloroethylene-induced rat kidney tumors: an investigation of the mechanisms involved and their relevance to humans. Toxicol Appl Pharmacol 103: 77–89

Guberan E, Fernandez J (1974) Control of industrial exposure to tetrachloroethylene by measuring alveolar concentrations: theoretical approach using a mathematical model. Br J Ind Med 31: 159–167

Hattis D, White P, Marmorstein L, Koch P (1990) Uncertainties in pharmacokinetic modeling for perchloroethylene. I. Comparison of model structure, parameters, and predictions for low-dose metabolism rates for models derived by different authors. Risk Anal 10: 449–457

Ings RM (1990) Interspecies scaling and comparisons in drug development and toxicokinetics. Xenobiotica 20: 1201–1231

International Commission on Radiological Protection (ICRP) (1975) Report of the Task Group on Reference Man—a report prepared by a Task Group of Committee 2 of the International Commission on Radiological Protection. Pergamon Press, Oxford, 480 pp

Koizumi A (1989) Potential of physiologically based pharmacokinetics to amalgamate kinetic data of trichloroethylene and tetrachloroethylene obtained in rats and man. Br J Ind Med 46: 239–249

Monster AC, Boersma G, Steenweg H (1979) Kinetics of tetrachloroethylene in volunteers; influence of exposure concentration and work load. Int Arch Occup Environ Health 42: 303–309

Mordenti J (1986) Man versus beast: pharmacokinetic scaling in mammals. J Pharm Sci 75: 1028–1040

Morgan A, Black A, Belcher DR (1970) The excretion in breath of some aliphatic halogenated hydrocarbons following administration by inhalation. Ann Occup Hyg 13: 219–233

Odum J, Green T, Foster JR, Hext PM (1988) The role of trichloroacetic acid and peroxisome proliferation in the differences in carcinogenicity of perchlorethylene in the mouse and rat. Toxicol Appl Pharmacol 92: 103–112

Opdam JJG (1989) Intra and interindividual variability in the kinetics of a poorly and highly metabolising solvent. Br J Ind Med 46: 831–845

Opdam JJG, Smolders JFJ (1986) Alveolar sampling and fast kinetics of tetrachloroethylene in man. 1—alveolar sampling. Br J Ind Med 43: 814–824

Racine-Poon A, Smith AF (1990) Population models. In: Berry DA (ed) Statistical methodology in the pharmaceutical sciences. Marcel Dekker, New York, pp 139–162

Reitz RH (1992) New data on tetrachloroethylene metabolism. OHEEA Advisory Panel Meeting. Cal-EPA, Berkeley

Sheiner LB (1984) The population approach to pharmacokinetic data analysis: rationale and standard data analysis methods. Drug Metab Rev 15: 153–171

Sheiner LB, Ludden TM (1992) Population pharmacokinetics dynamics. Annu Rev Pharmacol Toxicol 32: 185–209

Smith AFM (1991) Bayesian computational methods. Philos trans R Soc Lond, Series A 337: 369–386

Smith AFM, Wakefield JC (1994) The hierarchical Bayesian approach to population pharmacokinetic modelling. Int J Bio-Med Comput 36: 35–42

Tanner MA (1991) Tools for statistical inference—observed data and data augmentation methods. Springer, Berlin

Thomas A, Spiegelhalter DJ, Gilks WR (1992) BUGS: a program to perform Bayesian inference using Gibbs sampling. In: Bernardo JM, Berger JO, Dawid AP, Smith AFM (eds.) Bayesian statistics 4, Oxford University Press, Oxford, pp 837–842

Wakefield JC, Smith AFM, Racine-Poon A, Gelfand AE (1994) Bayesian analysis of linear and non-linear population models using the Gibbs sampler. Appl Stat—J R Statist Soc Series C 43: 201–221

Ward RC, Travis CC, Hetrick DM, Andersen ME, Gargas ML (1988) Pharmacokinetics of tetrachloroethylene. Toxicol Appl Pharmacol 93: 108–117

Williams LR, Leggett RW (1989) Reference values for resting blood flow to organs of man. Clin Phys Physiol Measure 10: 187–217

Woodruff TJ, Bois FY (1993) Optimization issues in physiological toxicokinetic modeling—a case study with benzene. Toxicol Lett 69: 181–196

Woodruff TJ, Bois FY, Auslander D, Spear R (1992) Structure and parametrization of toxicokinetic models: their impact on model predictions. Risk Anal 12: 189–201