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Precision and Sensitivity of Pharmacokinetic Models for Cancer Risk Assessment: Tetrachloroethylene in Mice, Rats, and Humans

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Precision and Sensitivity of Pharmacokinetic Models for Cancer Risk Assessment: Tetrachloroethylene in Mice, Rats, and Humans. BOIS, F. Y., ZEISE, L., AND TOZER, T. N. (1990). *Toxicol Appl. Pharmacol.* **102**, 300–315. Pharmacokinetic analyses have recently been incorporated in risk assessments, with resultant risks sometimes lower and associated "allowable" exposures higher, than would have been otherwise calculated. Predictions of coupled pharmacokinetic and multistage models, as used for regulatory purposes, are evaluated here for tetrachloroethylene carcinogenicity in mice, rats, and humans. Precision is studied by treating parameters as random variables and determining the range of risk estimates once parameter uncertainties are considered via Monte Carlo simulations. The methods developed in this study are of interest for any similar application. The resultant median risk estimate for humans exposed continuously to 1 ng/liter of tetrachloroethylene in the air is 1.6 per million and 5, 25, 75, and 95 percentiles are 0, 0.04, 2.8, and 6.8 per million. Sensitivity of the pharmacokinetic model predictions to its parameters is assessed by analyzing the results of the Monte Carlo simulations. The kinetic parameters defining the metabolic rate are the most important for the case studied.

The coupling of physiologically based pharmacokinetic models (PBPK) to multistage models of carcinogenesis has been recently used as a means to improve the assessment of risk from exposure to xenobiotics. Physiologic models describe the distribution and the biotransformation of chemicals and potentially provide estimates of the target tissue exposure to active carcinogens (e.g., rate of presentation of the active metabolites to the affected tissue). These models have been proposed to scale exposures among different species, different experimental conditions, or different doses (Andersen and Ramsey, 1983; Bischoff *et al.*, 1971; Clewell and Andersen, 1985; Gerlowski and Jain, 1983; Lutz *et al.*, 1984; Ramsey and Andersen, 1984; Ramsey and Gehring, 1980; Travis, 1987; Ward *et al.*, 1988).

To estimate the cancer risk in humans a dose–response model for carcinogenesis, for example the Crump multistage polynomial model (Anderson *et al.*, 1983; Crump, 1984) used for regulatory purposes, is needed to obtain a relationship for cancer risk as a function of target tissue exposure. Following the U.S. Environmental Protection Agency (EPA, 1986), the dose–response relationship obtained in animals is assumed to be the same for humans when exposure is expressed in terms of the carcinogenic metabolite(s) formation rate, scaled to the two-thirds power of the body weight. Typically, the uncertainties associated with model misspecification or parameter variability are not explicitly taken into account and the model output is assumed to provide exact estimates of risk. Quantitative knowledge of these uncertainties should aid the user of this approach to risk assessment in determining the confidence to be placed on the results (Bogen and McKone, 1988; Bogen and Spear, 1987; Bois

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et al., 1988, 1989; Hakanoglu *et al.*, 1985). In this paper, a statistical approach is used to evaluate the precision of a risk assessment based on the coupling of PBPK and multistage models. Because the parameter values of these models are not known with certainty, we describe them by probability distributions rather than by a fixed value. Monte Carlo sampling (Hammersley and Handscomb, 1964; Press *et al.*, 1986) over these distributions is used to generate a distribution of the risk. Results of a sensitivity analysis of the PBPK component to its parameters are also presented. Model bias is discussed in terms of more general assumptions pertaining to low dose and interspecies extrapolations.

For illustrative purposes, risk assessment is performed on tetrachloroethylene, a widely used volatile solvent which has been classified by the U.S. Environmental Protection Agency (EPA, 1985) as a probable human carcinogen and by the International Agency for Research on Cancer (IARC, 1979) as a known animal carcinogen. The cancer bioassay data used were from the National Toxicology Program (NTP, 1986) in which experiments were carried out in both sexes of B6C3F1 mice and F344/N rats.

METHODS

The Models

PBPK Model

The model used (Fig. 1) is the same as that of Fernandez *et al.* (1977), Droz and Guillemin (1983), Ramsey and Andersen (1984), and Andersen *et al.* (1987). The tissues are grouped as follows: fat, slowly perfused (muscles and skin), richly perfused (central nervous system and viscera, except liver), and metabolizing (liver). The model is mathematically described by a set of mass-balance differential equations. For tetrachloroethylene, metabolism is assumed to occur only in the liver and to be saturable, according to the Michaelis–Menten relationship. The formation of an epoxide by the cytochrome P450 system is a common step to the formation of the subsequent major metabolites and is assumed to be a saturable mechanism (Dekant, 1986). The epoxide, rather than the parent compound, is considered to be the "effective" or carcinogenically active form (EPA, 1985). As such, its daily rate of formation at steady state, or equivalently the rate of formation of metabolites, is taken as the measure of the exposure to the carcinogen. The same approach has been used by EPA (1985) and Bogen and McKone (1988).

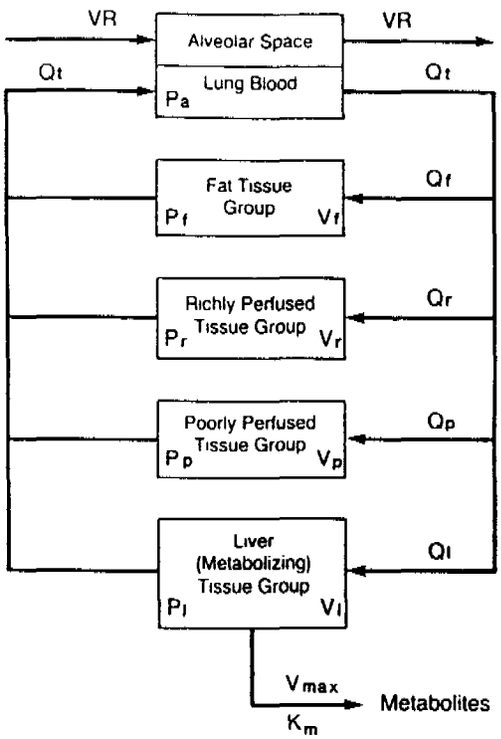

FIG. 1. Schematic representation of the physiological model used to simulate the distribution and metabolism of tetrachloroethylene. The symbols used are VR, alveolar ventilation rate; P_a, blood over air partition coefficient; Q_t, total blood flow; V_{max}, maximum rate of metabolism; K_m, Michaelis constant for the kinetic model of metabolism. Subscripts representing the tissues are used to identify the blood flows to the tissues, Q, the volumes, V, and the tissue over air partition coefficients, P. The units of the parameters are given in Table 1.

Multistage Model

The so-called linearized multistage, or Crump polynomial, model (Crump, 1984) is used to relate the lifetime probability or "risk" of cancer, $R(E)$, to the effective exposure, E, to the carcinogen, namely, the rate of formation of the epoxide or its surrogate measure, the rate of metabolism

$$R(E) = 1 - \exp(-\sum_{0}^{n} a_i \cdot E^i) \quad (1)$$

with $a_i \geq 0$ for all i.

The coefficients a_0 through a_n are estimated by maximum-likelihood analysis, using the program "Mstage" (Crouch, 1985). The NTP data for tetrachloroethylene allow the fitting of at most three parameters, so that n was fixed to 2 in our analysis. The coefficient a_1, or some statistical upper bound, is a measure of the carcinogenic activity of the chemical studied. It is defined as the "unit risk" by the EPA (Anderson *et al.*, 1983) and is similar to the "potency" of Crouch and Wilson (1981). "Extra risk," R, from exposure E is taken as the difference between the lifetime risk $R(E)$ and the background risk in the absence of exposure $R(0)$, relative to the fraction of the population expected to be cancer-free in the absence of exposure to the chemical (Anderson *et al.*, 1983; Crump, 1984), and is given by

$$R = \frac{R(E) - R(0)}{1 - R(0)}. \quad (2)$$

At low doses, extra risk then becomes

$$R \approx a_1 \cdot E. \quad (3)$$

PBPK Model Parameters and Their Probability Distributions

Parameter Estimates

The majority of physiologic and kinetic parameter values correlate with measures of body size (e.g., body weight and body surface area). Normalization of the parameters gives a scaling coefficient which, when multiplied by the measure, yields the parameter value. Table 1 lists such scaling coefficients and the appropriate multiplier (measure) for the parameters used. Median values for organ volumes, blood flows, and pulmonary ventilation rate are independent of the chemical in question. Partition coefficients were estimated from data obtained *in vitro* (EPA, 1986).

The values for V_{max} and K_m scaling coefficients were derived by fitting the PBPK model to *in vivo* data on rate of metabolite formation in response to tetrachloroethylene exposure. Statistical likelihood procedures were used: the likelihood function was obtained by tabulation under the assumption that the differences between predicted and experimentally observed values are normally distributed (Edwards, 1972). The V_{max} and K_m scaling coefficients corresponding to the maximum tabulated likelihood were taken as "maximum-likelihood estimates." During this fitting the other parameters were fixed to their median values.

For mice, V_{max} and K_m scaling coefficients values were estimated from the experiments of Buben and O'Flaherty (1985), Dekant *et al.* (1986), Mitoma *et al.* (1985), and Schumann *et al.* (1980). All exposures were by gavage, except for those of Schumann *et al.* in which an inhalation exposure was also assessed. The conditions and animal characteristics described in the studies were simulated. For simulating the data of Buben and O'Flaherty it was assumed that urinary metabolites represent 65% of the total metabolites and that this value is independent of the administered dose (EPA, 1986; Ward *et al.*, 1988). For rats, the experiments of Dekant *et al.* (1986), Frantz and Watanabe (1983), Mitoma *et al.* (1985), and Pegg *et al.* (1979) were used. In contrast to the other investigators, Dekant *et al.* (1986) used female animals.

For humans, the data used were from occupational surveys of Japanese female and male workers (Ikeda *et al.*, 1972; Ohtsuki *et al.*, 1983). Selected parameter values were adjusted, according to the anatomical model of Kerr *et al.* (1976), for differences in body weight and composition between average Japanese and American adults (Bogen and McKone, 1988). On the basis of experimental data in animals (Dekant *et al.*, 1986; Frantz and Watanabe, 1983; Mitoma *et al.*, 1985; Pegg *et al.*, 1979; Schumann *et al.*, 1980), the fraction of total metabolites recovered in the urine, in man, was assumed to be 65%.

Parameter Distributions

All the model parameters are subject to uncertainty. Parameter correlations must be taken into account, so that only physiologically realistic values are chosen. To do so we assigned probability distributions to the scaling coefficients (see Table 1), rather than to the parameters themselves. This ensured that the covariance structure imposed by the scaling is incorporated into the subsequent Monte Carlo simulations. For example, in the Monte Carlo simulations, liver volume and body weight are not allowed to vary independently, (i.e., with independent probability distributions); instead, the ratio of liver volume to body weight is allowed to vary within physiologically realistic values. The strong covariance of the estimates of the fitted kinetic parameters, V_{max} and K_m, was also taken into account. All the distributions were put in tabulated form for computation. The lower and upper limits of the scaling coefficient distributions are given in Table 1, except for V_{max} and K_m, for which reduction to one dimension was not appropriate (see Results). We now describe how these distributions were obtained.

Volumes. The probability distributions for the volume coefficients (percentages of the body weight) were derived from experimental data (S. Øie, University of California, personal communication). Individual measurements of liver volumes and body weight were obtained in 130 rats. The observed frequency distribution of their ratio was used to describe the shape of the percentage volumes for all organs and species.

TABLE 1

TABLE 1

PARAMETER SCALING COEFFICIENTS[a] AND THEIR VARIATION BOUNDS USED FOR TETRACHLOROETHYLENE IN MICE, RATS, AND HUMANS

		Mice		Rats		Humans		
Scaled parameter	Multiplier	Median	Bounds	Median	Bounds	Kerr[b]	EPA[c]	Bounds[d]
Body weight (Bw)	1	0.035	[0.03, 0.04]	0.35	[0.3, 0.4]	55	70	[30, 110]
Alveolar ventilation rate (Q_a)	$Bw^{0.74}$	0.19	[0.12, 0.27]	0.18	[0.11, 0.25]	0.26	0.15	[0.07, 0.22]
Total blood flow (Q_t)	$Bw^{0.74}$	0.24	[0.19, 0.30]	0.23	[0.18, 0.27]	0.27	0.19	[0.13, 0.24]
Blood flows								
Metabolizing tissue group (Q_l)	Q_t	0.37	[0.30, 0.45]	0.37	[0.30, 0.45]	0.25	0.37	[0.26, 0.48]
Fat tissue group (Q_f)	Q_t	0.09	[0.07, 0.11]	0.09	[0.07, 0.11]	0.04	0.09	[0.06, 0.12]
Richly perfused tissue group(Q_r)[e]	Q_t	0.42	—	0.42	—	0.52	0.42	—
Poorly perfused tissue group (Q_p)	Q_t	0.12	[0.09, 0.15]	0.12	[0.09, 0.15]	0.19	0.12	[0.08, 0.16]
Volumes								
Metabolizing tissue group (V_l)	Bw	0.04	[0.03, 0.05]	0.04	[0.03, 0.05]	0.02	0.02	[0.01, 0.03]
Fat tissue group (V_f)	Bw	0.09	[0.06, 0.11]	0.09	[0.06, 0.11]	0.15	0.20	[0.12, 0.28]
Richly perfused tissue group (V_r)	Bw	0.05	[0.03, 0.06]	0.04	[0.03, 0.05]	0.10	0.05	[0.03, 0.07]
Poorly perfused tissue group (V_p)[f]	Bw	0.72	—	0.73	—	0.63	0.63	—
Blood/air partition coefficient (P_a)	1	16.9	[13, 21]	18.9	[15, 23]	10.3	10.3	[6, 15]
Tissue/air partition coefficients								
Metabolizing tissue group (P_l)	1	70.3	[56, 84]	70.3	[56, 84]	38.3	38.3	[23, 54]
Fat tissue group (P_f)	1	2060	[1600, 2500]	2060	[1600, 2500]	1123	1123	[670, 1570]
Richly perfused tissue group (P_r)	1	70.3	[56, 84]	70.3	[56, 84]	38.3	38.3	[23, 54]
Poorly perfused tissue group (P_p)	1	20	[16, 24]	20	[16, 24]	10.9	10.9	[6.5, 15]
Maximum rate of metabolism (V_{max})[g]	$Bw^{0.7}$	0.06	—	0.017	—	0.007	0.007	—
Affinity constant (K_m)[g]	1	12.8	—	6.7	—	1.5	1.5	—
Intestinal absorption constant (K_t)[h]	1	0.01	—	0.01	—	0.01	0.01	—

Note. Scaled parameter = multiplier × scaling coefficient. Units: body weights in kg, flows (Q) in liter/min, volumes (V) in liters, V_{max} in mg/min, and K_m in mg/liter.

[a] Median values are from EPA (1986). In addition human values from Kerr *et al.* (1976) are listed. The V_{max} and K_m values are our own estimates (see text).

[b] These values were used to fit V_{max} and K_m from data obtained in Japan (see text). Volumes and flows are taken from the anatomical model of Kerr *et al.* (1976).

[c] These values were used in the Monte Carlo simulations to compute the rate of metabolism and the risk of cancer in the American adult (see text).

[d] These bounds apply to the human parameter values of the EPA (1986).

[e] Values for this parameter were computed at each simulation so that the sum of the blood flows was equal to 100% of the total flow.

[f] Values for this parameter were computed at each simulation so that the sum of the volumes was equal to 90% of the body volume.

[g] Median values are the maximum-likelihood estimates obtained in this study. The ranges of variation are defined by joint probability functions (see Results).

[h] This parameter was not varied in the Monte Carlo simulations as inhalation exposure was the only one simulated.

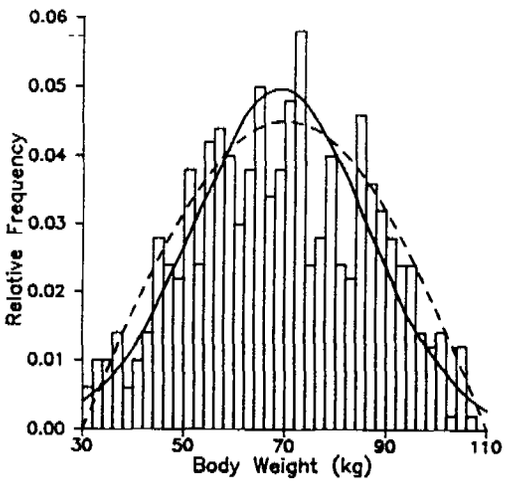

FIG. 2. Approximation of a truncated normal density function (solid line) by a cosine density function (dotted line), defined by the derivative of Eq. (4) (in the text). The histogram was constructed by random sampling of 500 values using the cosine function.

Body weight, flows, and partition coefficients. For these scaling coefficients, for which frequency distribution information is not readily available, we assumed the cumulative distribution function $F(x)$

$$F(x) = \frac{(1 - \cos(\pi \cdot x))}{2} \quad (0 \leq x \leq 1) \qquad (4)$$

for which

$$x = \frac{P - a}{b - a}. \qquad (5)$$

The parameter P is defined only over the closed interval $[a, b]$, to avoid negative and other unrealistic values. This function approximates a truncated normal distribution function (Fig. 2).

To define the bounds a and b, the body weights of mice and rats in the carcinogenicity study of the National Toxicology Program (NTP, 1986) were assumed to deviate by at most 15% from the average weight, at a given age. The average weight was approximately 35 g for mice and 350 g for rats during the NTP experiment. For humans the body weight had a median of 70 kg and bounds of 30 and 110 kg.

In the absence of good data on interindividual variability, the limits of flows and partition coefficients were best guesses from inquiries of animal experimentalists. For rats and mice, the scaling coefficient bounds for blood flows, ventilation rate, and partition coefficients were, ±20, ±40, and ±20% of the median value, respectively. For humans these bounds were ±30, ±50, and ±40% of the median value, respectively.

V_{max} and K_m. The distribution function for the scaling coefficients of these parameters is obtained from their likelihood function. The likelihood function, under tabulated form, was obtained for about 500 regularly spaced pairs of V_{max} and K_m scaling coefficient values during the search for the maximum-likelihood estimates.

The fitting procedure, the noise in the data, and the structure of the kinetic model result in both a correlation between the estimates of V_{max} and K_m and an error distribution (or uncertainty) for each of them. It is important to take these "statistical artifacts" into account. In fact, the covariance and error distribution of these estimates is exhaustively described by their joint, or bivariate, distribution function. This distribution function is proportional to the likelihood function of V_{max} and K_m, given the data observed (Edwards, 1972; Kalbfleisch, 1985). The proportionality constant must be such that the integral of the distribution function equals 1. Multiplication of the tabulated values by the appropriate constant gives the joint distribution function of the estimates.

Determination of Uncertainty in Risk Estimates

The probability distribution for risk estimates in humans is derived from probability distributions of

- the rate of metabolite formation in animals.
- the carcinogenic potency, expressed in terms of metabolite formation.
- the rate of metabolite formation in humans.

The distributions for metabolite formation were generated using the PBPK model, by Monte Carlo techniques, with runs involving 500 simulations. The uniform random number generator, provided with the Silicon Valley Software FORTRAN version 2.5, was used.

Distribution of the Rate of Metabolite Formation in Animals

The parameter values of the physiologic model were picked at random, according to their tabulated distributions. For V_{max} and K_m the tabulated joint distribution function was used. A linear interpolation between the tabulated points of the distribution functions was made for both the univariate and the joint distribution cases.

For both mice and rats, 500 sets of randomly selected parameter values were used to run the PBPK model and determine the average daily rate of formation of total metabolites for the two inhalation exposures in the NTP (1986) experiment (100 and 200 ppm in air for mice, and 200 and 400 ppm for rats, for 6 hr/day, 5 days/week). Each run represented 5 days of exposure. Following the EPA (1986), the rate of metabolism on the last day, multiplied by 5/7, was taken as an estimate of the average

steady-state value. Preliminary runs showed that this estimate does not deviate by more than a few percent from the average steady-state value obtained after 3 weeks of simulated exposure. The rates of metabolite formation were divided by the body weights to the two-thirds power, as done by EPA (1986) to normalize effective exposure to surface area. The same normalized rate was assumed for both sexes of mice or rats. In this way, 500 estimated pairs of effective exposure (formation of metabolites at steady state) were obtained, corresponding to the two inhalation exposures levels. These 500 pairs were used to construct a cumulative distribution function of the rate of formation of total metabolites for each exposure level.

Potency Distribution

Each one of the 500 pairs of exposure estimates, E, was used to generate a potency distribution by fitting the multistage model (Eq. (1)) to the cancer data (lifetime occurrence of hepatocellular tumors in male and female mice, and mononuclear cell leukemia for male and female rats). In this way 500 potency distributions were generated for each species and sex. These 500 distributions were convolved by Monte Carlo sampling to obtain the potency distribution for the species and sex considered. Thus, carcinogenic potency distributions were derived in terms of the daily rate of formation of total metabolites for rats and mice of each sex. As there is no evidence that any one of these potency distributions more accurately represents the picture in humans, they were convolved using Monte Carlo sampling to produce the cumulative distribution of potency for humans.

Distribution of Risk for Humans

As an example case the cancer risk to humans from a continuous exposure to 1 ng/liter of tetrachloroethylene in air was assessed. The distribution of the daily rate of formation of total metabolites, at steady state, was obtained by Monte Carlo simulations in the same fashion as it was for animals. Steady state was achieved in all simulations after 14 days of exposure. Following Eq. (3), the extra risk for humans is equal to the product of the rate of formation of total metabolites E by the carcinogenic potency a_1. The probability distribution of the risk was obtained by convolving the distributions of the rate of metabolism in humans and carcinogenic potency. This convolution was achieved by Monte Carlo sampling.

Sensitivity Analysis

The sensitivity of the pharmacokinetic model was studied by examining the correlation between the rate of metabolism, normalized for body surface area, and, for each physiologic parameter, the values generated during the Monte Carlo simulations. This analysis was performed for condition of low human exposure by continuous inhalation

RESULTS

Adjustment of V_{max} and K_m Scaling Coefficients

Maximum-likelihood estimates of V_{max} and K_m scaling coefficients obtained by fitting the full model to data for mice, rats, and humans are provided in Table 1. The contour plots of the joint distribution functions of these two parameters, for mice, rats, and humans, are presented in Fig. 3. In this figure the statistical correlation between V_{max} and K_m is evident: the major axes of the concentric shapes are not parallel to either the x- or the y-axis. Model predictions of the rate of metabolite formation, made by taking the maximum-likelihood estimates for V_{max} and K_m, and median values for the other parameters are compared to observed rates in Fig. 4. On average, model predictions do not deviate by more than 22% from the observations. As an example, the maximum-likelihood fit of the model to the data of Buben and O'Flaherty (1985) is presented in Fig. 5. The predictions fit the data well.

Frequency Distributions

Figure 6 presents the results of the Monte Carlo simulations for the rate of metabolite formation in mice and rats after exposures to tetrachloroethylene.

In mice, the median rate of metabolite formation is 7.3 mg/day/kg$^{2/3}$ (with 5 and 95 percentiles of 5.7 and 9.3 mg/day/kg$^{2/3}$) at 100 ppm. On doubling the exposure to 200 ppm the median rate increases to a value of 12.7 mg/day/kg$^{2/3}$ (with 5 and 95 percentiles of 10.4 and 15.3 mg/day/kg$^{2/3}$).

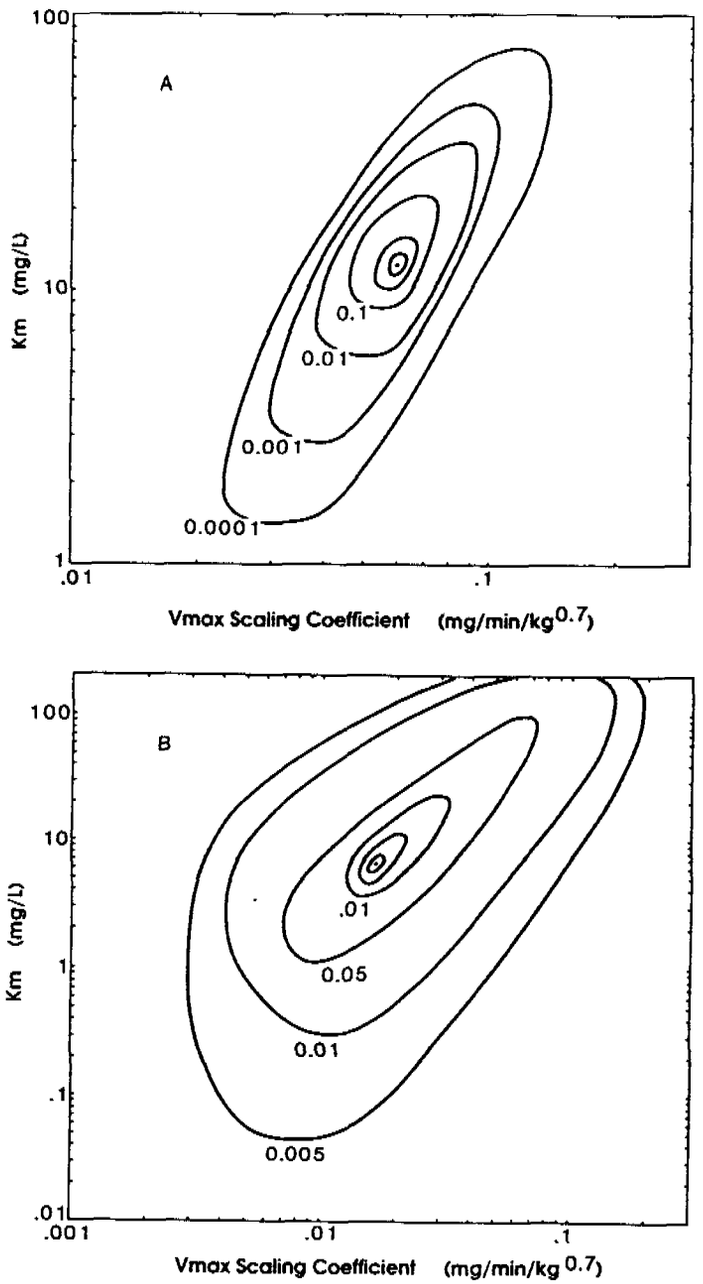

FIG. 3. Contour plots of the joint distribution functions obtained for V_{max} and K_m in mice (A), rats (B), and humans (C). The concentric curves define the conditional confidence intervals for the two parameters and are labeled according to their p values. The two innermost curves define the 20% ($p = 0.2$) and 50% ($p = 0.5$) confidence intervals. The shapes were smoothed using spline interpolation.

In rats, the median rate of metabolite formation is 9.8 mg/day/kg$^{2/3}$ (with 5 and 95 percentiles of 6.9 and 12.4 mg/day/kg$^{2/3}$) at 200 ppm. At 400 ppm exposure, the median rate of formation increases to a value of 13.9 mg/day/kg$^{2/3}$ (with 5 and 95 percentiles of

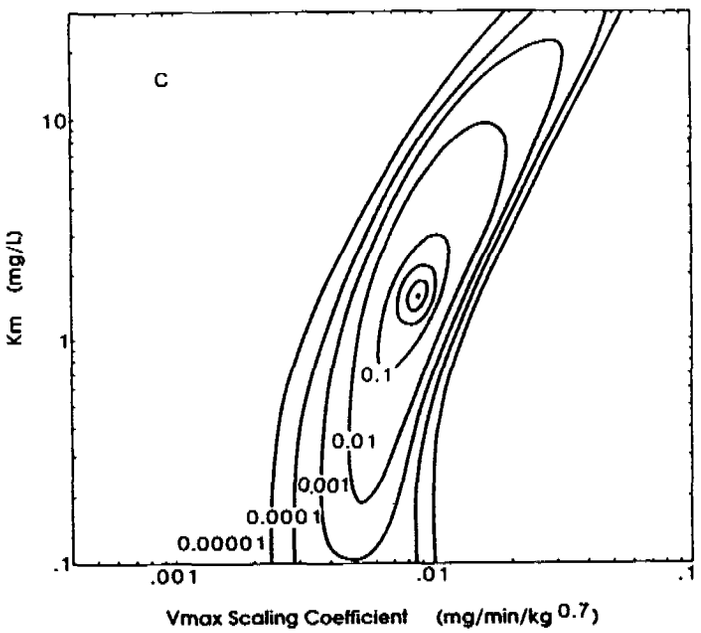

FIG. 3—*Continued*

11.8 and 16.8 mg/day/kg$^{2/3}$). In both mice and rats the medians of metabolite formation rates increase with the rate of exposure toward a limiting value (saturable metabolism).

The frequency distributions of the carcinogenic potency, for mice and rats of both sexes, and their convolved distribution are presented in Fig. 7. After convolution the median potency value is 0.032 (mg/day/kg$^{2/3}$)$^{-1}$ with 5 and 95 percentiles of 0 and 0.11 (mg/day/kg$^{2/3}$)$^{-1}$.

Figure 8 shows the distribution obtained for the rate of formation of total metabolites in humans, for a continuous exposure to 1 ng/liter of tetrachloroethylene. The rate of metabolism at steady state (after surface area correction) has a median of 58 ng/day/kg$^{2/3}$ and 5 and 95 percentiles of 34 and 104 ng/day/kg$^{2/3}$. Due to the covariance of V_{max} and K_m estimates, the variability of the rate of formation of metabolites is much lower than might be expected from the variability of these parameters (the 5 and 95 percentiles of V_{max} and K_m marginal distributions were 0.07 and 0.18 mg/min and 0.4 and 2.1 mg/liter, respectively). Figure 9 presents the cumulative distribution of the predicted cancer risk for humans. The corresponding frequency distribution is skewed and shows a median of 1.6 per million with 5, 25, 75, and 95 percentiles of 0, 0.04, 2.8, and 6.8 per million, respectively. These results take into account variation in both the rate of formation of the total metabolites and the carcinogenic potency.

Model Sensitivity

The results of the sensitivity analysis are presented in Table 2. It appears that the model predictions of steady-state rates of metabolite formation are particularly sensitive to changes in K_m, V_{max}, and the partition coefficients. Examples of representative scatterplots are shown in Fig. 10.

DISCUSSION

Precision

The analyses presented here address the degree to which the precision of risk estimates

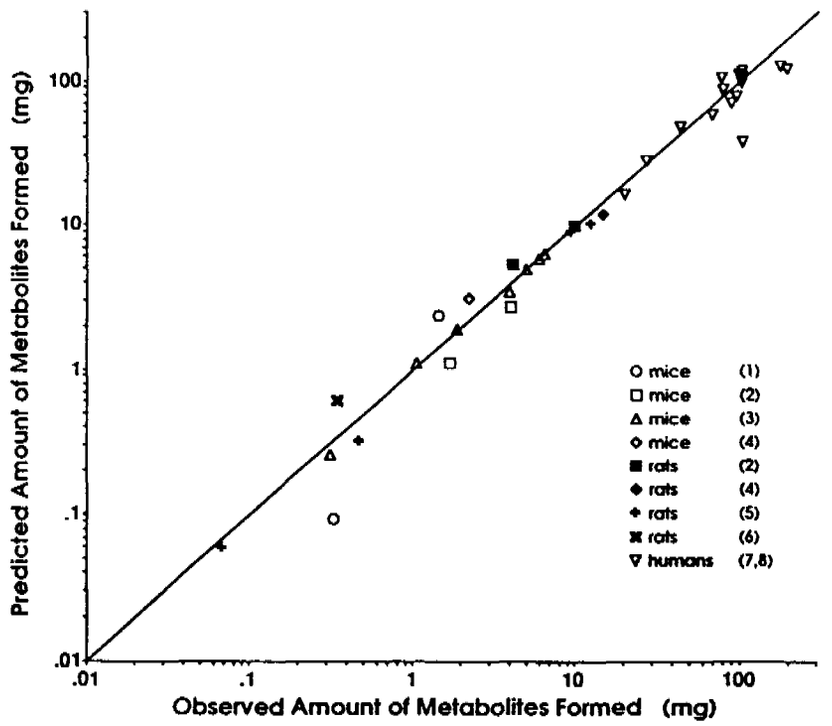

FIG. 4. Model predictions based on maximum-likelihood estimates of V_{max} and K_m are compared to *in vivo* observations for rats, mice, and humans. The numbers between parentheses in the legend correspond to the following references: (1) Schumann *et al.* (1980); (2) Mitoma *et al.* (1985); (3) Buben and O'Flaherty (1985); (4) Dekant *et al.* (1986); (5) Pegg *et al.* (1979); (6) Frantz and Watanabe (1983); (7) Ikeda *et al.* (1972); (8) Ohtsuki *et al.* (1983).

is influenced by uncertainty in parameter values. This precision can be described by the frequency distribution of the risk estimates and may be somewhat underestimated here, due to several simplifications that were made in specifying the distribution functions of the physiologic model parameters.

First, we took into account the interdependencies among parameters (e.g., organ volumes and blood flow rates) by scaling to body size. The relationships used to scale parameters were assumed to be known with certainty. This assumption leads to an underestimate of the variance associated with the parameters of the model. Yet, sensitivity analysis, as discussed below, shows that uncertainty in the parameters, other than V_{max} and K_m, has a limited effect on the results.

Second, in the absence of suitable data, certain parameter distributions were based on the judgement of expert researchers. For blood flows, ventilation rates, and partition coefficients, an idealized symmetric distribution was used, which may not be realistic.

Third, although, for V_{max} and K_m, a joint distribution function was obtained, which describes adequately the major part of their variability and interaction, these parameters were assumed not to covary with the partition coefficients or the rate of intestinal absorption. No data were available to assess such covariances. Measures of exhalation rate after oral gavage would allow a statistical adjustment of the oral absorption rate and a more extensive study of the covariance structure of the model. Furthermore, only limited data

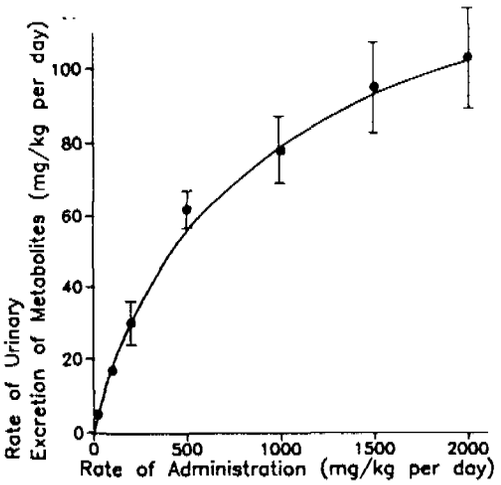

FIG. 5. Relationship between the oral rate of administration of tetrachloroethylene and the rate of formation of metabolites as assessed by the rate of their urinary excretion in mice (Buben and O'Flaherty, 1985). The model predictions (solid line) fit the data well. The means (±SE) at each rate of administration assayed are shown.

sets with few dose groups or individuals were available for fitting V_{max}, K_m, and the parameters of the multistage model. The distributions of estimates for these parameters may not correspond to their distribution in a large population. For example, V_{max} and K_m values for humans were derived from data in a racially homogeneous population of healthy adult male workers (Ikeda *et al.*, 1972; Ohtsuki *et al.*, 1983). Although other data in humans are available (Monster *et al.*, 1979; Monster, 1979; Monster and Houtkooper, 1979), they were not used, because only the urinary excretion of trichloroacetic acid was monitored, when the data of Ikeda and Ohtsuji (1972) show that other metabolites may also be excreted. We decided to use the total metabolites in that trichloroacetic acid alone would have underestimated the rate of metabolism of tetrachloroethylene in humans.

Finally, there is an error associated with the use of any finite number of Monte Carlo runs (in particular in the estimates of the tails of the distributions). With 500 runs, the errors in the percentiles reported here should be relatively small.

Sensitivity

The relationship between the rate of metabolism and the estimates of V_{max} shows paradoxical behavior: the rate decreases when V_{max} increases (Fig. 10c), when just the opposite is expected. This is due to the fact that V_{max} and K_m estimates are statistically correlated. The form of the correlation, described by the joint distribution function shown on Fig. 3c, implies that the ratio, V_{max}/K_m (to which the rate of metabolism is proportional at the low dose studied), increases more rapidly than V_{max}. Choosing at random a high V_{max} in the Monte Carlo simulations implies selecting a K_m such that, on average, the ratio V_{max}/K_m and the corresponding rate of metabolism are lower than when a low V_{max} is picked. For example, for the rat data shown in Fig. 3b: when V_{max} is sampled around 0.1, K_m is most likely, given the data, to be in the neighborhood of 2 (V_{max}/K_m = 0.05), but when V_{max} is around 0.2, K_m is at about 10,

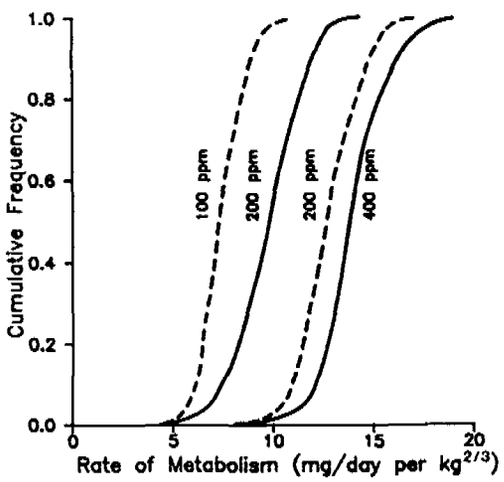

FIG. 6. Monte Carlo-generated cumulative distribution of the rate of metabolite formation (mg/day/kg$^{2/3}$) in mice (dotted lines, 100 and 200 ppm exposures) and rats (solid lines, 200 and 400 ppm exposures). Exposure conditions of the NTP (1986) inhalation bioassays for tetrachloroethylene were simulated. To obtain the rate in units of mg/day these results may be multiplied by the body weight to the two-thirds power.

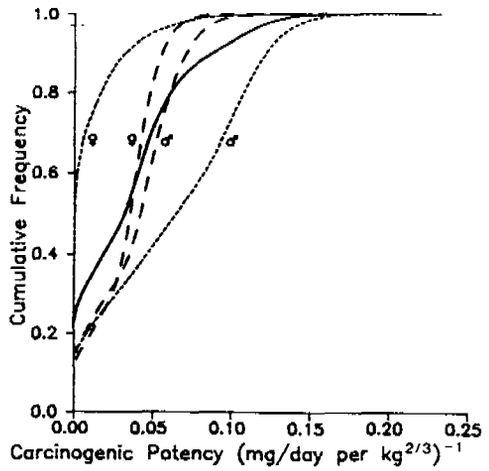

FIG. 7. Monte Carlo-generated cumulative distributions of the carcinogenic potency of tetrachloroethylene in male and female mice (dotted lines) and rats (dashed lines) and the potency distribution (solid line) obtained by convolution of the preceding ones. NTP (1986) cancer data were used to fit the multistage model. The variability in potency incorporates the expected variability in the rates of metabolite formation (cf. Fig. 6).

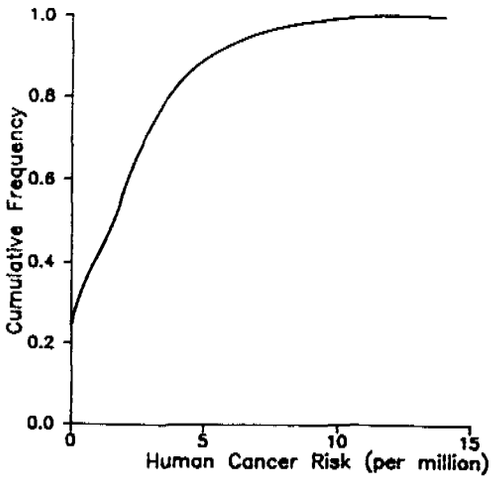

FIG. 9. Cumulative distribution of the human cancer risk estimate associated with a continuous inhalation exposure to 1 ng/liter of tetrachloroethylene.

on average, so that $V_{max}/K_m = 0.02$. The ratio drives the behavior of the model at low exposure, this is why V_{max}/K_m and the rate of metabolism tend to decrease when V_{max} increases.

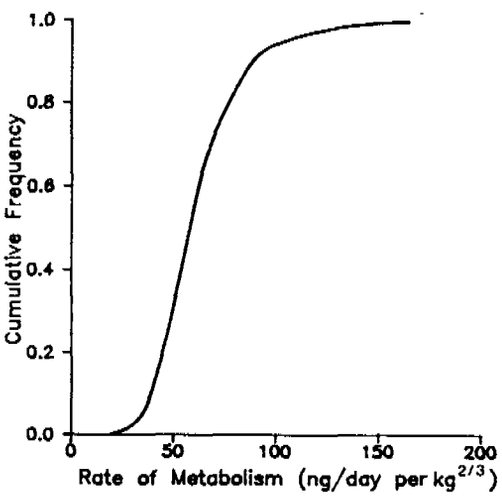

FIG. 8. Monte Carlo-generated cumulative distribution of the rate (mg/day/kg$^{2/3}$) of metabolite formation in humans. A continuous inhalation exposure to 1 ng/liter of tetrachloroethylene was simulated.

The sensitivity of the model results to the choice of parameter values was assessed by examining their one-to-one correlations (Table 2, Fig. 10). Low correlations indicate model insensitivity to the parameter selection (for example, at steady state, following low inhalation exposure, the model is insensitive to the values of blood flows, alveolar ventilation rate, and organ volumes). On the other hand, high correlations may be due to covariance between parameters and not necessarily to a high sensitivity of the model. Only part of the covariance structure of the parameters was implemented, namely the dependence on body weight and the covariance between V_{max} and K_m estimates. The lack of relevant data prevented us from obtaining a better description of the correlations among parameters. Due to these limitations, the high figures presented in Table 2 may be somewhat misleading. However, the major features exhibited by this approximation are likely to be valid in this case.

The sensitivity analysis indicates that the kinetic model may be simplified without loosing any appreciable accuracy in the

TABLE 2

CORRELATION COEFFICIENTS OF THE RATE OF METABOLITE FORMATION WITH THE PARAMETERS OF THE PHARMACOKINETIC MODEL AS OBSERVED DURING THE MONTE CARLO SIMULATIONS OF TETRACHLOROETHYLENE EXPOSURE IN HUMANS

Parameter	Correlation coefficient[a]
Body weight	0.042
Alveolar ventilation rate	0.184
Total blood flow	0.040
Blood flows	
Metabolizing tissue group	0.046
Fat tissue group	0.017
Poorly perfused tissue group	0.061
Volumes	
Metabolizing tissue group	0.045
Fat tissue group	−0.016
Richly perfused tissue group	0.006
Blood/air partition coefficient	0.391
Tissue/air partition coefficients	
Metabolizing tissue group	−0.237
Fat tisue group	−0.329
Richly perfused tissue group	−0.287
Poorly perfused tissue group	−0.225
Maximum rate of metabolism (V_{max})	−0.466
Affinity constant (K_m)	−0.727

[a] The absolute value of the correlation coefficient for a parameter increases with the sensitivity of the model to this parameter.

model predictions. The multiple body compartments can be replaced by a single one with reversible pulmonary exchange and Michaelis–Menten metabolism. Sensitivity analysis of the complete model is a means of checking the validity of such simplifications.

Bias

Errors due to misspecification of the PBPK or cancer models are likely to be many times larger than those due to parameter selection, particularly at low dose. Because low-dose data on metabolism and cancer risk are not available in both humans and animals, the models cannot be fully validated and their biases directly assessed.

We chose to investigate the behavior of the multistage polynomial model of carcinogenesis, the one most commonly used by regulatory agencies. Bias in the predictions of this model has been discussed by a number of authors (e.g., Freedman and Zeisel, 1988; Zeise *et al.*, 1987). Major sources of bias occur in both the relationship between exposure and response and the measure of exposure. There may be multiple mechanisms of carcinogenesis for tetrachloroethylene, some of which are unlikely to be adequately described by the multistage polynomial. Furthermore, large interspecies differences, even for a given cancer mechanism, may be present. For example, peroxisome proliferation may have a role in the induction of liver tumors by tetrachloroethylene in mice, whereas this may not be true for rats (Dekant, 1986; Odum *et al.*, 1988). The use of the rate of formation of total metabolites (assumed to be proportional to the rate of formation of an epoxide) as a measure of effective exposure can be questioned. Other possible measures would be, for example, the cumulative exposure to the carcinogens, as suggested by Dedrick (1981), or the concentration of active metabolites in target tissues.

It was assumed that both the hepatocellular tumors in mice and the mononuclear cell leukemias in rats were equally indicative of cancer risk in humans. Under this assumption, the convolution of the potencies obtained from the four bioassay data sets gives a measure of potency in humans less biased than the value derived from selecting the most sensitive species.

Another issue here is whether the use of the PBPK model results in better estimates of effective exposure. A host of simplifications are made and several potentially nonlinear phenomena were not considered. For example, in the physiologic model presented, it is assumed that metabolism occurs in the liver and that the active metabolite is too unstable to be transported far, and yet leukemia is observed in the rat. Furthermore, detoxification reactions are not taken into account; the pos-

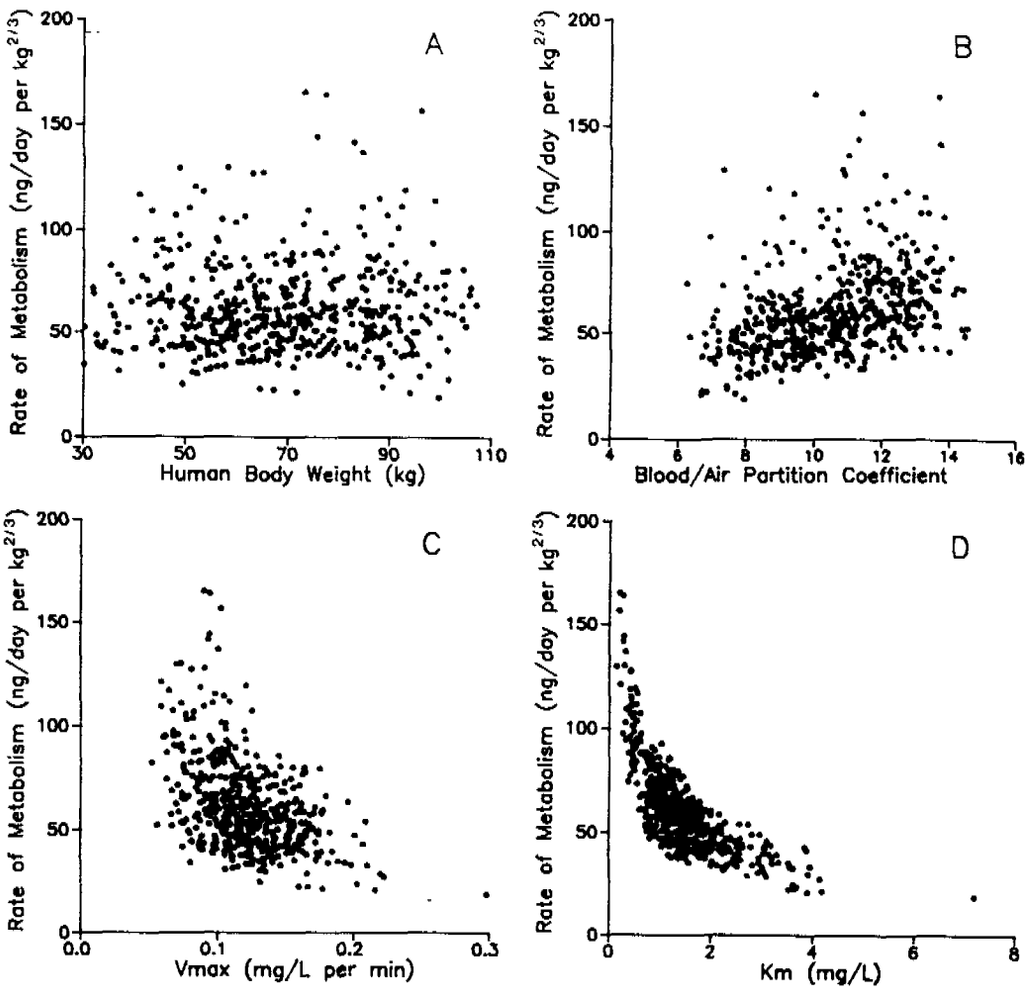

FIG. 10. Plot of the rate of metabolism of tetrachloroethylene (ng/day/kg$^{2/3}$) in humans, observed during the Monte Carlo simulations, versus randomly generated values of (A) body weight; (B) blood over air partition coefficient; (C) maximum rate of metabolism (V_{max}); (D) affinity constant (K_m). The correlation coefficients are 0.042, 0.39, 0.47, and 0.73, respectively. Large absolute values of the correlation coefficient indicate a high sensitivity.

sibility of autoinduction of metabolism is not examined; important cellular (and pharmacokinetic) processes, such as DNA repair or DNA methylation, are ignored; *in vitro* data on partition coefficients for homogenized tissues are assumed to be representative of the situation *in vivo;* and only a single enzymatic reaction following Michaelis–Menten kinetics is assumed. It should be noted that unlike Ward *et al.* (1988), we included no parallel linear pathway for tetrachloroethylene metabolism. For two reasons, there is no convincing evidence of its contribution to the total elimination of tetrachloroethylene, and a good fit of the metabolic data, even for mice, can be obtained without such a component (Fig. 5). Sex specificity in tetrachloroethylene metabolism was not considered explicitly in the model: the fit to the animal data (Fig. 4) did not point to large differences between the

sexes, so that data on female and male animals were pooled. For humans, the available data do not allow for the investigation of gender differences. Finally, general procedures for extrapolating metabolic parameters or carcinogenic potency from one species to another have not been validated. In this respect the validity of surface area normalization for the effective exposure, in addition to a correction for the metabolic parameters, can be questioned. We adopted this normalization procedure in order to enable the comparison of our results with EPA risk assessment for tetrachloroethylene.

Little is known about the importance of these simplifications or the biases that they introduce. The direction (under- or overestimation) of the bias is frequently not certain. All these simplifications are due to our ignorance of the processes involved, our inability to describe them adequately, and a lack of data. To account for these biases, the approach we have taken may be extended by incorporating some procedures of decision analysis.

CONCLUSION

The approach developed here has many potential applications. Monte Carlo simulations are a powerful tool for studying the behavior of complex biomathematical models. Likelihood techniques allow for a rigorous description of parameter covariance, such a description being essential during the Monte Carlo sampling. Sensitivity analysis gives further information on the importance of each parameter and offers a convenient way of checking for possible model simplifications. Finally, this approach can also be used to identify those parameters which may require additional experimentation.

As a result of the use of coupled pharmacokinetic and multistage models of carcinogenesis, a median cancer risk estimate of 1.6 per million was determined for humans continuously exposed to 1 ng/liter of tetrachloroethylene in air. Taking the uncertainty in the model parameters into account yields, for the same exposure level, values for the 5, 25, 75, and 95 percentiles of 0, 0.04, 2.8, and 6.8 per million. Because the experimental data, in particular in humans, are still inadequate, these percentiles may not be accurate. Nevertheless, they point to the precision that might be expected from regulatory risk assessment procedures, if the models used are assumed to be correct. Unfortunately, as discussed, the biases introduced by an improper choice of models could be many times larger than the errors due to the variability of the parameters. Considerable time may elapse before scientific understanding of the processes involved allows for the confident use of any particular model of carcinogenesis. The use of pharmacokinetics is an area in which considerable improvement over the current approach might be achieved. Our results emphasize that elucidation of both the metabolic processes and the mechanism of carcinogenesis is of primary importance.

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