AMERICAN INDUSTRIAL HYGIENE ASSOCIATION JOURNAL 57:23–32 (1996)

Variability in Biological Exposure Indices Using Physiologically Based Pharmacokinetic Modeling and Monte Carlo Simulation

AUTHORS
Russell S. Thomas
Phillip L. Bigelow
Thomas J. Keefe
Raymond S.H. Yang

Center for Environmental Toxicology & Technology
Department of Environmental Health, Colorado State University, Fort Collins, CO 80523

By using physiologically based pharmacokinetic (PBPK) modeling coupled with Monte Carlo simulation, the interindividual variability in the concentrations of chemicals in a worker's exhaled breath and urine were estimated and compared with existing biological exposure indices (BEIs). The PBPK model simulated an exposure regimen similar to a typical workday, while exposure concentrations were set to equal the ambient threshold limit values (TLV®s) of six industrial solvents (benzene, chloroform, carbon tetrachloride, methylene chloride, methyl chloroform, and trichloroethylene). Based on model predictions incorporating interindividual variability, the percentage of population protected was derived using TLVs as the basis for worker protection. Results showed that current BEIs may not protect the majority or all of the workers in an occupational setting. For instance, current end-expired air indices for benzene and methyl chloroform protect 95% and less than 10% of the worker population, respectively. Urinary metabolite concentrations for benzene, methyl chloroform, and trichloroethylene were also estimated. The current BEI recommendation for phenol metabolite concentration at the end-of-shift sampling interval was estimated to protect 68% of the worker population, while trichloroacetic acid (TCAA) and trichloroethanol (TCOH) concentrations for methyl chloroform exposure were estimated to protect 54% and 97%, respectively. The recommended concentration of TCAA in urine as a determinant of trichloroethylene exposure protects an estimated 84% of the workers. Although many of the existing BEIs considered appear to protect a majority of the worker population, an inconsistent proportion of the population is protected. The information presented in this study may provide a new approach for administrative decisions establishing BEIs and allow uniform application of biological monitoring among different chemicals.

The assessment of human exposure to industrial solvents is a difficult and complex problem for industrial hygienists who seek to evaluate and control factors relating to worker health. Traditionally, industrial hygienists have relied on air monitoring techniques to estimate human exposures, while the relative safety of the worker is evaluated through comparisons with reference standards. However, the monitoring of airborne concentrations suffers from several shortcomings that have been outlined and discussed previously.[1,2] Primarily, airborne concentrations of the chemical may not reflect the actual absorbed dose due to pharmacokinetic factors or other routes of absorption.

In 1981 reference standards for chemical substances in biological media were adopted by the American Conference of Governmental Industrial Hygienists (ACGIH) to encourage an integrated

This research was supported, in part, by research contract (F33615-91-C-0538) from the Toxicology Division, Armstrong Laboratory, U.S. Air Force, a Superfund Basic Research program project grant (P42 ES05949) from the National Institute of Environmental Health Sciences, and a research contract (F49620-94-1-0304) from the Air Force Office of Scientific Research.

Copyright 1996, American Industrial Hygiene Association

approach for assessing chemical exposures. These standards define limits on the amounts of substances (or their metabolites) to which a worker may be exposed without demonstrable hazard to health or well-being as determined from the worker's tissues, body fluids, or exhaled breath.[3] Consequently, biological exposure indices (BEIs) represent the biological levels of determinants that are collected from a worker who has been exposed to an airborne chemical at the threshold limit value (TLV®) and, logically, provide the same margin of safety as TLVs.

For the purpose of establishing BEIs, physiologically based pharmacokinetic (PBPK) models have been employed to relate airborne concentrations of chemicals to the levels of the parent compound or their metabolites in a variety of body fluids or tissues.[4,5] In physiologically based pharmacokinetic models, the body is subdivided into anatomical compartments representing individual organs or tissue groups. The movement of chemicals between the compartments is described by mass balance differential equations that incorporate blood flows, partition coefficients, and tissue volumes. After incorporating metabolic and potential pharmacodynamic processes, the fate and disposition of the chemical and metabolites can be predicted and extrapolated to a variety of exposure scenarios. Although physiological models have been successfully applied to the description of industrial chemicals within the human body, the primary weakness in using BEIs derived from physiologically based pharmacokinetic modeling is the variability within the human population;[6] specifically, human variability diminishes the relationship between absorbed dose and biological indicators.

In an attempt to address this problem, Droz et al.[7,8] created a "population-based" physiological model in which selected physiological and exposure parameters were assigned realistic statistical distributions. The model predictions of expired air and urinary metabolite concentrations were compared with the results of field studies to evaluate the reliability of biological indicators. However, the model utilized by Droz et al.[7,8] did not incorporate many of the physiological and pharmacokinetic principles currently employed in PBPK models. Current PBPK models differ from classical or conventional pharmacokinetics in that (1) they utilize a large body of physiological and physiochemical data that are not chemical-specific; (2) they afford, with more confidence, intraspecies and interroute extrapolation; and (3) they may be used to predict *a priori*, from limited data, the pharmacokinetic behavior of certain chemicals.[9] This study attempts to expand on the work of Droz et al.[7,8] by combining current PBPK models with statistical simulation techniques (i.e., Monte Carlo simulation). The linkage of PBPK models with Monte Carlo simulation allows the assessment of interindividual variability by conducting pharmacokinetic studies computationally on a very large number of humans (in this case n = 1000 humans) with varying physiological and metabolic parameters. A detailed description of coupling PBPK models with Monte Carlo simulation is described elsewhere.[10]

The objectives of this study were to (1) assess the variability in biological exposure indices for benzene, carbon tetrachloride, chloroform, methyl chloroform, methylene chloride, and trichloroethylene using PBPK modeling and Monte Carlo simulation; and (2) compare the current BEIs listed in the ACGIH handbook[11] with the model-derived values.

MATERIALS AND METHODS

Model Structure

The basic physiological model structure is described by Leung[5] and illustrated in Figure 1. The body is subdivided into five tissue groups consisting of the liver, lung, slowly perfused, rapidly perfused, and fat, while each compartment is represented by mass balance differential equations that incorporate blood flows, partition coefficients, and tissue volumes. The concentration of the chemical of interest in the blood leaving the lung is assumed to be in equilibrium with the concentration in alveolar air as determined by the blood:air partition coefficient. The chemical is distributed to all tissues and eliminated by metabolism in the liver, as well as by exhalation. Excretion of metabolites is assumed to occur through a first-order process (K_e), and the fractional metabolite formation (F_m) is included for phenol, trichloroacetic acid, and trichloroethanol. The mass balance equation for the liver can describe metabolism in terms of a single saturable process, a first-order process, or a combination of the two. Specifically, the saturable process has a maximum metabolic rate, V_{max} (mg/hr), and a Michaelis constant, K_m (mg/liter). The first-order constant, K_f, has units of hr^{-1}. A description of the mass-balance differential equations used in the model is presented in detail elsewhere.[5]

Physiological values used in the present model are scaled as a function of body weight or cardiac output.[12,13] It should be noted that many pharmacokinetic studies rely on interspecies (i.e., cross-species) scaling of alveolar ventilation and cardiac output as a function of body surface area (e.g., body weight raised to the two-thirds power). However, intraspecies (i.e., within species) scaling factors are used in this model and calculated as a direct function of body weight:[14]

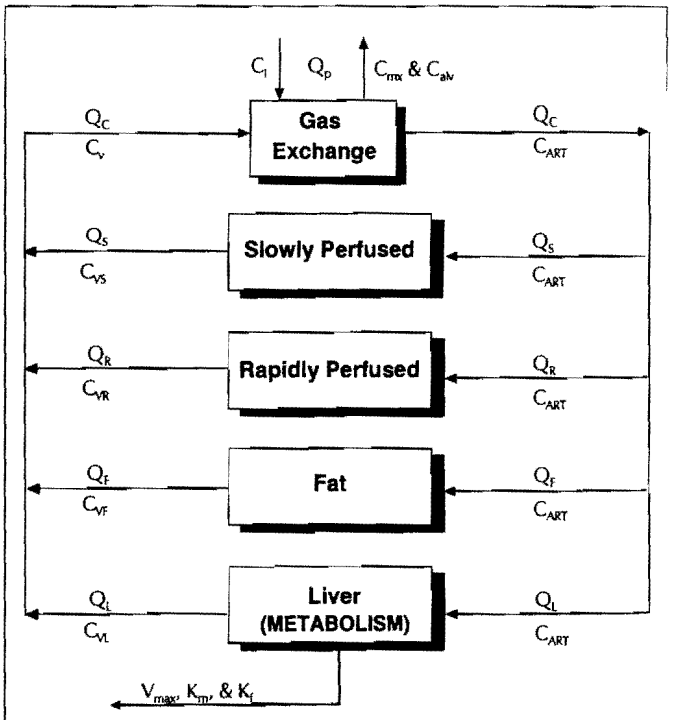

FIGURE 1. Diagram of the physiologically based pharmacokinetic model utilized for the six chemical solvents. Body tissues are grouped into four compartments: slowly perfused, rapidly perfused, fat, and liver. The solvents enter the body through inhalation (C_I) with absorption into pulmonary blood occurring in the gas-exchange compartment. Concentrations of the chemical in the venous blood (C_v) and arterial blood (C_{art}) are calculated based on blood flow (Q_i) and solubility information. After incorporating metabolic processes, such as Michaelis-Menton metabolism (V_{max} and K_m) or first-order enzymatic conjugation (K_f), fate and disposition of the chemical can be calculated for predicting concentrations in exhaled air (C_{mx} and C_{alv}) and/or urine.

$$Q_p = Q_{pc} * BW \quad (1)$$

where Q_p is the alveolar ventilation (L/hr), Q_{pc} is the unscaled ventilation (L/hr kg), and BW is the body weight in kg. Blood flows to the liver, richly perfused, slowly perfused, and fat were calculated as a percentage of cardiac output, while tissue volumes were calculated as a percentage of body weight. Metabolic and solubility parameters were used directly as model input and not scaled to body weight.

Chemical input to the model (C_{inh}) was set equal to the TLV concentration of the associated chemical.[5,11] As stated previously, BEIs represent the concentration of the chemical or metabolite collected from a worker who has been exposed to an airborne concentration at the TLV. Therefore, the duration of exposure was constructed to simulate an 8-hour workday per day for five days per week, and the worker was assumed to perform light-duty exercise (50 W) during the 8-hr workday, followed by 16 hours of rest prior to the next shift. The concentration in mixed expired air (C_{mx}) was calculated by assuming that two-thirds of the exhaled air was alveolar (C_{alv}).[5]

The basic model structure was modified for Monte Carlo simulation by resampling model parameters within assigned distributions. Consequently, central tendencies and associated dispersions were assigned to each model parameter. The central tendencies were obtained from Leung[5] while the dispersions were acquired from a variety of published studies. It was assumed that the intraindividual variability, or variability within the same worker from day to day, was relatively small in comparison to interindividual or between worker variability.[7,8] Intraindividual variability was, therefore, neglected for simplicity. Unless otherwise indicated, all percentages expressed in the Materials and Methods section are coefficients of variation.

Variability in Model Flow Parameters

A summary of estimates for the interindividual variability in physiological flow parameters is outlined in Table I. Alveolar ventilation was measured as a function of age and activity in subjects clinically free of pulmonary and cardiovascular disease.[15] While no significant differences for interindividual variability were observed with age, variability during exercise was consistently higher (25 to 29%) than that found at rest (10 to 19%). This suggests that the individual variability normally associated with physical conditioning may not be manifested at basal conditions to the extent found during exercise. As a result, the variability associated with alveolar ventilation in the physiological model during resting and light work conditions was assigned to be 20 and 30%, respectively.

Variability of cardiac output in resting subjects was similar to that reported for alveolar ventilation.[16-19] Variability ranged from 15% in 15 males of unreported age[17] to 22% in 11 females at 24 years of age.[16] Measurements during physical activity were reported by Holmgren et al.[19] for subjects riding a bicycle ergometer at 300 kpm/min, but the interindividual variability in those measurements was not significantly different from that observed at rest. In contrast, studies by Mitchell et al.[17] and Chapman and Fraser[16] revealed variability changes in cardiac output among subjects during exercise. Specifically, Mitchell et al.[17] observed a 24% variability in 15 males walking on a treadmill, while subjects at rest possessed a variability of only 15%. Chapman and Fraser[16] reported variabilities for male and female subjects during exercise of 25% and 42%, respectively. Consequently, variability in cardiac output was assumed to increase with exercise and estimated to be equal to that of alveolar ventilation.

The variability associated with liver blood flow was considerably greater than that reported for cardiac output during resting conditions. Using the clearance of radioactive xenon as an indicator, investigations by Lundbergh and Strandell[20] and Sherriff et al.[21] produced variability estimates of nearly 30%. However, the variability of liver blood flow is compounded by individual variability in cardiac output. Therefore, the uncertainty reported by Lundbergh and Strandell,[20] as well as by Sherriff et al.,[21] is a composite

TABLE I. Summary of Estimates for Interindividual Variability in Physiological Flow Parameters Used in Monte Carlo Simulation

PBPK Parameter	No. of Subjects	Sex	Mean Age (yrs)	Coefficient of Variation (%) Resting	Coefficient of Variation (%) Exercise	Reference No.
Flows:						
Alveolar	[A]	M	25.5	10	25[B]	15
	[A]	M	42.7	19	28[B]	15
	[A]	M	59.6	14	29[B]	15
Cardiac output	23	M	25.2	15	25[C]	16
	11	F	24.2	22	42[C]	16
	15	M	[D]	15	24[E]	17
	12	M	30.3	18	—	18
	18	M, F	21.3	17	17[F]	19
Liver	10	M	24.8	28	—	20
	14	[A]	[A]	28	—	21
Rapidly perfused	9	M	50.1	22[G]	—	22
	9	M	[A]	17[H]	—	23
Slowly perfused	15	M	25.1	—	56, 40, 29[I]	24
	10	M, F	32.8	20[J]	—	25
	10	M, F	32.8	50[K]	—	25
Fat	18	M	43.5	26	—	26
	14	F	45.3	19	—	26

[A] Not stated in article
[B] Exercise consisted of stepping up on platform (20 cm high) and down again at a rate of 30 cycles/minute
[C] Exercise consisted of walking on treadmill at 3 mph on 5% grade
[D] Age range reported from 20–50 yrs
[E] Exercise consisted of walking on a treadmill at maximum oxygen uptake
[F] Exercise consisted of riding a bicycle ergometer at 300 kpm/minute
[G] Cerebral blood flow
[H] Kidney blood flow
[I] Quadriceps' blood flow during bicycle ergometer workout at 16–28, 40–51, and 62–88% of maximal oxygen uptake, respectively
[J] Cutaneous blood flow at ambient temperature of 19–22 °C
[K] Subcutaneous blood flow at ambient temperature of 19–22 °C

of liver blood flow and cardiac output variabilities. Consequently, an estimate of the independent variability of liver blood flow was derived using a standard method in the propagation of uncertainty represented by the following equation:[27]

$$CV_{tissue+cardiac} = \sqrt{CV^2_{cardiac} + CV^2_{tissue}} \quad (2)$$

where $CV_{tissue+cardiac}$ is the coefficient of variation for the tissue and cardiac output combined, while $CV_{cardiac}$ and CV_{tissue} are the coefficients for cardiac output and tissue alone. In the current model, the total variability associated with liver blood flow and cardiac output was estimated to be 30% based on the xenon clearance studies, while the variability associated with cardiac output was estimated to be 20%. Based on Equation 2, a coefficient of variation of 22% was calculated for liver blood flow alone.

The variability associated with slowly perfused and fat blood flows are compounded by the same problems associated with the liver blood flow. Estimates of variability associated with fat blood flow in men and women (which include cardiac output variability) have been reported as 26% and 19%, respectively.[26] Applying the same methods of uncertainty propagation and assuming a coefficient of variation of 25% for the fat blood flow plus cardiac variability, the uncertainty associated with fat blood flow alone was calculated to be 15%.

For variability in slowly perfused blood flow, studies involving cutaneous and muscle blood flow measurements were used. Whereas variability in cutaneous and subcutaneous blood flow has been reported to be 20 and 50%, respectively,[25] Grimby et al.[24] found that variability in blood flow to the muscles that comprise the quadriceps decreased as a function of exercise intensity. Specifically, variability in blood flow to the quadriceps during bicycle ergometer workouts of progressive intensities dropped from 56 to 29%,[24] but the consistent decrease in individual variability can be explained through physiological blood flow autoregulation. As the intensity of the exercise increases, skeletal muscles exhibit autoregulatory characteristics in which blood flow is regulated metabolically at the local level and, therefore, removed from changes in cardiac output.[28] Although the methods of uncertainty propagation no longer apply during increasing activity, the individual variability associated with cardiac output must be accounted for during resting conditions. Therefore, assuming a variability of 50% in the combined slowly perfused and cardiac output, the uncertainty for slowly perfused blood flow alone was calculated to be 45%.

In contrast to blood flow to the fat and skeletal muscles, blood flow to the majority of organs assigned to the richly perfused compartment is autoregulated even at basal levels.[28] Therefore, the variability estimates reported for cerebral and kidney blood flows of 22 and 17%,[22,23] respectively, were not adjusted for the variability in cardiac output. Variability in richly perfused blood flow within the model was estimated to be 20%.

Variability in Model Volume Parameters

A summary of estimates for the interindividual variability in physiological volume parameters is summarized in Table II. With the exception of body weight and fat volume, the interindividual variability in tissue volumes was generally consistent at 20%. As a result, liver and rapidly perfused tissue volumes were estimated to vary 20% in the model.

The variability in fat tissue was reported in a study by Womersley et al.[33] of sedentary and "muscular" men and women between the ages of 16 and 49 years. The coefficients of variation for men and women were approximately 30 and 20%, respectively. Therefore, model variability in fat tissue weights was conservatively assumed to be 30%.

TABLE II. Summary of Estimates for Interindividual Variability in Physiological Volume Parameters Used in Monte Carlo Simulation

PBPK Parameter	No. of Subjects	Sex	Mean Age (yrs)	Coefficient of Variation (%)	Reference No.
Weights:					
Body	21,752	M, F	39	13	29
Liver	38	M	25	21	30
	54	M	35	20	30
	58	M	45	19	30
	39	M	55	15	30
Rapidly perfused	3	M, F	52	13[B]	31
	[A]	M	25	21[C]	32
	[A]	F	25	23[C]	32
Fat	12	M	23.6	31[D]	33
	12	M	21.1	32[E]	33
	12	F	21.9	16[D]	33
	12	F	27.2	22[E]	33

[A] Not stated in article
[B] Variability for lung weights
[C] Variability for heart weights
[D] Sedentary men or women
[E] "Muscular" men or women engaged in competitive athletics

Variability in Model Metabolic Parameters

A summary of the variability in metabolic and excretion parameters is presented in Table III. The variability in V_{max} and K_m was determined primarily through *in vitro* studies by Peter et al.[34] and Reitz et al.,[35] but the focus of the information on variability was limited to the cytochrome P-4502E1 isozyme due to its role in metabolizing the solvents in this study.[39–41] Variability in V_{max} and K_m was reported to be 44% and 18%, respectively, using chlorzoxazone as a probe for cytochrome P-4502E1.[34] In contrast, Reitz et al.[35] reported considerably larger variations for V_{max} (73%) and K_m (41%) using methylene chloride as a substrate. Reitz et al.[35] attributed the large variation in metabolic activity to a single liver sample with abnormally high activity. When the value for the spurious liver sample was removed from the analysis, variability in V_{max} and K_m was 57% and 18%, respectively. As expected, the interindividual variability in V_{max} is considerably greater than the variability associated with K_m. This is attributed to the individual variability in chemical intake, namely ethanol, which is known to induce cytochrome P-4502E1.[41] Induction of this isozyme would greatly affect the maximum metabolic rate of the enzyme (V_{max}) while having little effect on enzyme affinity (K_m). The *in vitro* variability information was supported by immunohistochemical studies by Wrighton and Stevens[42] who found a variability of 44% in immunodetectable cytochrome P-4502E1 among 14 human liver samples. As a result, V_{max} and K_m were assigned model variabilities of 50% and 20%, respectively.

The variability in first-order metabolic activity (K_f) was estimated from studies by Hunter et al.,[37] Datta et al.,[36] and Reitz et al.[3] However, of the solvents studied only methylene chloride has been demonstrated to possess a significant first-order conjugation activity.[43] Datta et al.[36] reported a coefficient of variation of 15% in the bromsulphthalein-glutathione conjugation activity in 10 human biopsy specimens, while Reitz et al.[35] observed a 67% variability in glutathione S-transferase (GST) activity using methylene chloride as a substrate. However, the high coefficient of variation in the Reitz

TABLE III. Summary of Estimates for Interindividual Variability in Physiological Metabolic and Excretion Parameters Used in Monte Carlo Simulation[A]

PBPK Parameter	No. of Subjects	Sex	Mean Age (yrs)	Coefficient of Variation (%)	Reference No.
Metabolism:					
V_{max}	14	[B]	[B]	44	34
	4	[B]	[B]	73[C]	35
K_m	14	[B]	[B]	18	34
	4	[B]	[B]	41[C]	35
K_f	4	[B]	[B]	67[D]	35
	10	[B]	[B]	15	36
	21	[B]	[B]	26	37
F_m	20	[B]	[B]	25–45	7
Excretion:					
Creatinine	7	M	49	32	38
Urine volume	9	M	49	16[E]	38

[A] Variability in metabolic parameters determined by *in vitro* analysis
[B] Not stated in article
[C] The high coefficient of variation was skewed due to an outlying liver sample. When the sample was removed, the coefficients of variation for V_{max} and K_m were 57 and 18%, respectively.
[D] The high coefficient of variation was skewed due to a liver sample reporting no glutathione-S-transferase activity
[E] Mean urinary volume measurements over a 24-hr period

et al.[35] study was skewed due to a liver sample reporting no GST activity. When the value for that sample was omitted, a coefficient of variation of only 8% was calculated. Investigation of another phase II enzyme system, UDP-glucuronyltransferase (UDP-GT), was performed by Hunter et al.[37] in which the concentration of D-glucaric acid was measured from the inhibitory effect of glucarolactone on β-glucuronidase. A variability of 26% was observed in UDP-GT activity within a group of 21 control subjects.[37] Considering the wide range of uncertainty estimates, a conservative value of 30% was used for K_f model variability.

Variability in Model Excretion and Solubility Parameters

Variability estimates for creatinine excretion and urinary volume were obtained from a study of nine male lead workers whose renal function and serum creatinine concentrations were considered normal[38] (Table III). Between-subject variability in urinary volume excretion over a 24-hour period was calculated to be 16%, while creatinine variability was reported at 32%.[38] Although uncertainty in urinary parameters may vary with degree of hydration and level of activity, model variability in creatinine excretion and urinary volume was estimated to be 30% and 20%, respectively. Variability in the first-order excretion of urinary metabolites (K_e) was also assumed to be 30%.

Phenol excretion as an indicator of benzene exposure was adjusted for background levels of phenol based on a study of 40 urine samples by van Haaften and Sie.[44] The variability in urinary phenol concentrations was reported to be 64% with a mean of approximately 6.6 mg/L of urine. Solubility parameters were obtained from Gargas et al.,[45] and the standard deviations were calculated based on the standard errors and the number of samples (Table IV) reported by Gargas.[46]

Parameter Distributions and Constraints

The statistical distributions for model parameters were assumed to be either lognormal or normal[47] and are summarized in Tables V and VI. With the exception of body weight and slowly perfused blood flow, the distributions of model parameters were truncated at ±3 standard deviations to eliminate any outliers that would not remain within the bounds of physiological constraints. It should be noted that fractional flow, metabolic, and volume parameters were truncated at ±3 standard deviations or constrained between 0 and 1 to maintain mass balance. Body weight was not truncated, and the blood flow to slowly perfused tissues was truncated at ±2 standard deviations due to its high variability (45%). Although metabolic parameters, such as V_{max}, also possessed relatively high variability, they were truncated at ±3 standard deviations due to the inherent ability of the human population to induce enzymatic function. Three standard deviations was chosen after considering the ranges reported in the literature listed above.

For model stability, as well as for practical reasons, alveolar ventilation was set equal to cardiac output during resting conditions, and the sum of the individual organ masses was constrained to be less than the total body mass. This was achieved by setting the volume of slowly perfused tissue equal to the difference between body mass and remaining tissues. A conservation of blood flow during resting conditions was accomplished by setting the flow to rapidly perfused tissues equal to the difference between cardiac output and flows to remaining compartments. During light work activity, the flow to slowly perfused tissues was set to equal the difference between cardiac output and remaining flows. A total of 1000 simulations was performed for each solvent using the SimuSolv™ (Dow Chemical, Co., Midland, Mich.) software package; the distribution algorithms were obtained from Naylor et al.[48]

TABLE IV. Summary of Estimates for Tissue: Air Solubility Parameters Used in Physiological Pharmacokinetic Model and Monte Carlo Simulations[A,B,C]

Chemical	Blood:Air	Liver:Air	Fat:Air	Richly Perfused:Air	Slowly Perfused :Air
Benzene	8.19 (0.17)	17.0 (2.6)	499 (24)	17.0 (2.6)	10.3 (1.8)
Carbon tetrachloride	2.73 (0.46)	14.2 (2.0)	359 (21)	14.2 (2.0)	4.57 (1.18)
Chloroform	6.85 (1.02)	21.1 (2.96)	203 (9)	21.1 (2.96)	13.9 (4.7)
Methylchloroform	2.53 (0.26)	8.60 (2.38)	263 (34)	8.60 (2.38)	3.15 (0.81)
Methylene chloride	9.70 (0.07)	14.2 (2.4)	120 (12)	14.2 (2.4)	7.92 (3.54)
Trichloroethylene	8.11 (0.34)	27.2 (10.2)	554 (42)	27.2 (10.2)	10.1 (6.6)

[A] Partition coefficients collected from Gargas et al.[45]
[B] Sample sizes used to calculate standard deviations obtained from Gargas[46]
[C] Values represent mean (SD)

Data Treatment

Statistical analysis of the biological concentrations was performed using standard methods based on normal theory via the Minitab software program (Minitab, Inc., State College, Pa.). Normality of each data set was statistically assessed using Filliben's proce-

dure.[49] A one-sided normal percentile was calculated according to the following equation:

$$BC_{lower} = \mu - Z * \sigma \quad (3)$$

where BC is the biological concentration at the lower normal percentile (i.e., for the lower 5th percentile, 95% of all values would be higher), μ is the estimated mean, σ is the estimated standard deviation, and Z is the standard normal value corresponding to the cumulative probability and desired percentile (e.g., 1.645 for the lower 5th percentile). In the event that a transformation was needed to achieve normality, the BC values were computed using the transformed variable, and Equation 3 was followed by the appropriate back-transformation.

RESULTS

Theoretical Basis For Deriving "Protected Population"

BEIs represent the levels of biological determinants likely to be observed in specimens collected from a worker exposed to an airborne chemical at the TLV.[1] In turn, TLVs represent the time-weighted average concentration for a normal 8-hour workday and a 40-hour workweek, to which a worker may be repeatedly exposed, day after day, without adverse effect.[11] Unless external factors intervene (e.g., dermal absorption, ingestion, nonoccupational exposure, or personal habits of the workers), BEIs afford the same margin of safety as TLVs.[1] Consequently, TLVs were employed as the "gold-standard" and used as the input concentration for the PBPK model (see Materials and Methods). The theoretical basis, therefore, is based on the protection afforded by the TLV.

Summary Statistics

The results of the model predictions for expired air concentrations are described in Tables VII and VIII. Of the solvents studied, all six had significant concentrations in the expired air at the end-of-shift sampling, which suggests that expired air concentrations can be a suitable biological index if the type of expired air (end or mixed) is specified.

Comparisons with current expired air recommendations were possible for benzene and methyl chloroform at the prior-to-next shift and prior-to-last shift sampling times. The current BEI for benzene in end-exhaled air is listed as 0.12 ppm[11] and was estimated by the model to protect 95% of the worker population (Table VII). In contrast, the current BEI of methyl chloroform in end-exhaled air (40 ppm)[11] was much lower than the corresponding model predictions and protects, in fact, less than 10% of the worker population (Table VII).

Model variability in urinary metabolite concentrations is summarized in Table IX. The current BEI recommendation for phenol metabolite concentration with benzene exposure at the end-of-shift sampling time was 50 mg/g creatinine.[11] Based on model predictions, the current BEI recommendation for phenol in urine protects an estimated 68% of the worker population.

Methyl chloroform and trichloroethylene determinants were estimated from the trichloroacetic acid (TCAA) and trichloroethanol (TCOH) concentrations. Model predictions of TCAA and TCOH for methyl chloroform exposure at the lower 5th percentile were 2.9 and 37 mg/g creatinine, respectively. As a result, the recommended level for TCAA concentration (10 mg/g) was estimated to protect 54% of the worker population while the TCOH recommendation (30 mg/g) protects 97%[11]

TABLE V. Model Parameters Used in PBPK/Monte Carlo Simulation to Determine Variability in Biological Exposure Indices

PBPK Parameter	Sample Dist.[A]	Form[B]	At Rest Mean	At Rest CV (%)	During Light Work Mean	During Light Work CV (%)
Weights						
Body (kg)	L	—	70.0	13	—	—
			Percentage of Body Weight			
Liver	N	B	0.03	20	—	—
Rapidly perfused	N	B	0.06	20	—	—
Slowly perfused	—	B	[C]	—	—	—
Fat	N	B	0.23	30	—	—
Flows						
Alveolar (L/hr)	L	S	4.97	20	18.9	30
Cardiac output (L/hr)	L	S	[D]	—	8.61	30
			Percentage of Cardiac Output			
Liver	N	C	0.25	22	0.16	22
Rapidly perfused	N	C	[E]	—	0.27	20
Slowly perfused	N	C	0.19	45	[E]	—
Fat	N	C	0.05	15	0.06	15
Metabolic						
V_{max} (mg/hr)	L	—	[F]	50	—	—
K_m (mg/L)	L	—	[F]	20	—	—
K_f (hr^{-1})	L	—	[F]	30	—	—
F_m	L	—	[F]	45	—	—
Excretion						
K_e (hr^{-1})	L	—	[F]	30	—	—
Creatinine (mg/L)	N	—	1.35	30	—	—
Urinary vol. (L/8 hrs)	L	—	0.52	20	—	—

[A] Sample distributions obtained from Portier and Kaplan[47] (N = normal, L = Lognormal)
[B] Computational forms: B = percentage of body weight, S = scaled as a function of body weight (L/hr kg), C = percentage of cardiac output
[C] Slowly perfused tissue weight set to the difference of body weight and remaining tissues (see text)
[D] Cardiac output set to equal alveolar ventilation
[E] Tissue blood flow set to the difference of cardiac output and remaining tissue flows (see text)
[F] Mean values vary according to chemical (see Table VI)

TABLE VI. Mean Values of Metabolic Model Parameters Used in Physiological Pharmacokinetic Model and Monte Carlo Simulations[A]

Chemical	V_{max} (mg/hr)	K_m (mg/L)	K_f (hr^{-1})	K_e (hr^{-1})	F_m
Benzene	29.04	0.35	—	0.2	70
Carbon tetrachloride	12.72	0.25	—	—	—
Chloroform	307.2	0.45	—	—	—
Methylchloroform	8.22	5.75	—	0.026, 0.0069[B]	75, 25[B]
Methylene chloride	118.8	0.58	0.53[C]	—	—
Trichloroethylene	195.7	0.25	—	0.026, 0.0069[B]	45, 32[B]

[A] Mean parameter values obtained from Leung[51]
[B] First-order excretion constants (K_e) and fractional metabolite formation (F_m) for trichloroethanol and trichloroacetic acid, respectively
[C] Of the solvents studied, only methylene chloride has been demonstrated to possess significant first-order conjugation activity (see text)

(Table IX). Finally, the recommended concentration of TCAA in urine as a determinant of trichloroethylene exposure is 100 mg/g creatinine,[11] which protects an estimated 84% of the workers (Table IX).

DISCUSSION

Although mathematical models with biologically interpretable parameters are becoming more prevalent in estimating BEIs, the current practice does not take into consideration the intrapopulation variability of these parameters. If some model parameters have large intrapopulation variability, then deterministic models may produce estimates of biological indicators that do not protect the majority of the worker population despite the protection afforded through the TLV.

According to ACGIH, phenol concentrations in the urine are only to be applied to a group of workers, rather than to an individual worker, due to the large variability in background urine concentrations.[50] As a result, a direct comparison of model-derived results and the recommended levels can be deceiving. Instead, the recommended concentration of 50 mg/g creatinine should, theoretically, compare with the model estimate protecting 50% of the worker population. According to model estimates the recommended phenol concentration protects an estimated 68% of the population and, when applied to a group of individuals according to ACGIH recommendations, should provide adequate protection.

As a supplement to urinary phenol, the monitoring of exhaled air is recommended for workers exposed to benzene.[50] The current BEI recommendations of 0.08 and 0.12 ppm for mixed- and end-exhaled air collected prior to next shift,[11] respectively, protect an estimated 95% of the worker population. However, the interindividual variability in expired air concentrations is compounded by differences in physical activity during and after exposure. Therefore, reducing the potential for variations in physical activity would result in a decrease in the total uncertainty. Since concentrations of benzene in exhaled air rise sharply at the beginning of the exposure and reach steady state within 1 to 2 hours,[50] a BEI based on the expired air concentrations at the end-of-shift sampling time may provide a more suitable index.

Three biological determinants are recommended to monitor methyl chloroform exposure (i.e., methyl chloroform, TCAA, and TCOH). Specifically, methyl chloroform in exhaled air prior-to-shift and TCAA in urine are generally considered as indicators of integrated exposures on the monitoring day and previous days while TCOH in urine is a better indicator of recent exposure.[50] According to modeling results, the current BEI for end-exhaled air is estimated to protect less than 10% of the worker population, while metabolite recommendations for urinary TCAA and TCOH are estimated to protect 54% and 97%, respectively. Significant disparity, therefore, exists among the biological indicators relative to the proportion of population protected. Furthermore, results from model estimates of exhaled breath and TCAA suggest that workers are not being adequately protected with respect to current indicators of integrated exposure.

Biological monitoring of trichloroethylene exposure is also performed using the presence of TCAA and TCOH metabolites in urine. However, current ACGIH recommendations include a combined TCAA and TCOH concentration of 300

TABLE VII. Variability in PBPK Model Predicted End-Exhaled Air Concentrations as Compared with Current Biological Exposure Indices[A]

Chemical	Current BEI (ppm)	Predicted End-Exhaled Air Concentrations (ppm) Percent of Population Protected 95%	80%	50%
Benzene				
End-of-shift	—	5.7	6.3	7.0
Prior-to-next-shift	0.12	0.12	0.16	0.22
Prior-to-last-shift	—	0.30	0.38	0.49
Carbon tetrachloride				
End-of-shift	—	4.0	4.2	4.4
Prior-to-next-shift	—	0.02	0.03	0.05
Prior-to-last-shift	—	0.06	0.10	0.14
Chloroform				
End-of-shift	—	4.9	5.6	6.4
Prior-to-next-shift	—	0.11	0.16	0.21
Prior-to-last-shift	—	0.18	0.24	0.31
Methyl chloroform				
End-of-shift	—	311	318	326
Prior-to-next-shift	—	2.4	3.3	4.7
Prior-to-last-shift	40	7.3	9.4	12.2
Methylene chloride				
End-of-shift	—	27	31	34
Prior-to-next-shift	—	0.37	0.76	1.2
Prior-to-last-shift	—	0.36	0.84	1.3
Trichloroethylene				
End-of-shift	—	29	32	35
Prior-to-next-shift	—	0.56	0.78	1.1
Prior-to-last-shift	—	1.5	1.9	2.5

[A] End-of-shift = shortly before end of an 8-hr exposure. Prior-to-next-shift = before the second shift of five-day workweek after 8-hr exposure and 16-hr rest. Prior-to-last-shift = before fifth shift of five-day workweek after four consecutive 8-hr exposures and 16-hr rests.

TABLE VIII. Variability in PBPK Model Predicted Mixed-Exhaled Air Concentrations as Compared with Current Biological Exposure Indices[A]

Chemical	Current BEI (ppm)	Predicted Mixed-Exhaled Air Concentrations (ppm) Percent of Population Protected 95%	80%	50%
Benzene				
End-of-shift	—	7.1	7.5	8.0
Prior-to-next-shift	0.08	0.08	0.11	0.15
Prior-to-last-shift	—	0.20	0.25	0.33
Carbon tetrachloride				
End-of-shift	—	4.4	4.5	4.6
Prior-to-next-shift	—	0.02	0.02	0.03
Prior-to-last-shift	—	0.04	0.07	0.09
Chloroform				
End-of-shift	—	6.6	7.1	7.6
Prior-to-next-shift	—	0.08	0.10	0.14
Prior-to-last-shift	—	0.12	0.16	0.21
Methyl chloroform				
End-of-shift	—	324	329	334
Prior-to-next-shift	—	1.6	2.2	3.2
Prior-to-last-shift	—	4.9	6.3	8.1
Methylene chloride				
End-of-shift	—	35	37	40
Prior-to-next-shift	—	0.25	0.51	0.78
Prior-to-last-shift	—	0.24	0.56	0.89
Trichloroethylene				
End-of-shift	—	36	38	40
Prior-to-next-shift	—	0.37	0.52	0.73
Prior-to-last-shift	—	1.0	1.3	1.7

[A] End-of-shift = shortly before end of an 8-hr exposure. Prior-to-next-shift = before the second shift of five-day workweek after 8-hr exposure and 16-hr rest. Prior-to-last-shift = before fifth shift of five-day workweek after four consecutive 8-hr exposures and 16-hr rests.

mg/g creatinine, as well as TCAA concentration alone (100 mg/g creatinine).[11] Although the current TCAA recommendation is estimated to protect 84% of the worker population, the combined TCAA and TCOH recommendation would protect greater than 95% of the worker population, since the TCOH concentration alone is greater than 400 mg/g creatinine at 95% (Table VIII).

By combining the tools currently afforded the toxicology and pharmacokinetic fields (i.e., PBPK modeling and Monte Carlo simulation), the establishment of standards designed for worker protection can be carried out with less uncertainty. Specifically, the addition of realistic variability information to established PBPK models may provide a consistent basis for administrative decisions concerning BEIs. While many existing BEIs addressed in this study appear to protect a majority of the worker population, an inconsistent proportion of the population is protected. Therefore, reevaluation of current indicators according to "population-based" BEIs may be necessary to allow uniform and reliable application of biological monitoring among different chemicals.

Of the solvents presented in this study, only carbon tetrachloride is believed to have the potential for significant dermal absorption as indicated by the skin notation in the ACGIH handbook.[11] Although no attempt was made to consider dermal exposure in this model, previous efforts by McDougal et al.[51,52] have incorporated dermal absorption into PBPK models for a variety of organic chemicals. Future efforts could combine dermal and inhalation exposures into a PBPK model (with Monte Carlo simulation) to determine the "population-based" BEIs for multiroute exposures.

Reliance on the TLV as the "gold-standard" for establishing inhalation BEIs is a weakness of the present model that needs to be addressed. Recent advances in molecular and biochemical toxicology have permitted an understanding of the toxic mechanisms underlying many of the common industrial chemicals. From a predictive standpoint, mechanistic information can be incorporated into what are presently called pharmacodynamic models. In contrast to pharmacokinetic models that describe what the body does to the chemical, pharmacodynamic models characterize what the chemical does to the body. The real potential lies in the ability to link the two types of models in order to quantitate the target tissue dose of the reactive chemical or its metabolite and describe the associated biological effects. Together with Monte Carlo simulation, physiologically based pharmacokinetic and pharmacodynamic modeling (PBPK/PD) can provide a mechanistic basis from which to establish not only BEIs, but also TLVs. A PBPK/PD model for the hepatotoxicity of carbon tetrachloride, alone and following kepone pretreatment, has been developed and evaluated by El-Masri et al.[10]

Further research associated with PBPK/PD modeling may also address issues associated with mixed chemical exposures. According to Fiserova-Bergerova,[1] correlation between intensity of exposure and biological levels may be disrupted by interference of another chemical or even its own metabolites.[53] These disruptions can occur on the pharmacokinetic[54] or pharmacodynamic level and may result in interactions appreciably different than the additive adjustment currently recommended by ACGIH.[11] For example, kepone, an organochlorine pesticide used for fire ant control, has been found in the environment.[55] At environmentally realistic levels (e.g., 10 ppm in the diet), kepone can produce as high as a 67-fold increase in lethality compared to an otherwise marginally toxic dose of carbon tetrachloride.[10,55,56] Therefore, the concept of chemical mixtures must be addressed in exposures both inside and outside the workplace with PBPK/PD models incorporating toxicologic interactions on the mechanistic level.

TABLE IX. Variability in PBPK Model Predicted Urinary Metabolite Concentrations as Compared with Current Biological Exposure Indices[A]

Chemical and Metabolite	Current BEI (mg/g creatinine)	Predicted Urinary Metabolite Concentrations (mg/g creatinin Percent of Population Protecte 95%	80%	50%
Benzene				
Phenol[B]	50	27	41	6
Methyl chloroform				
Trichloroacetic acid	10	2.9	5.7	1
Trichloroethanol	30	37	63	11
Trichloroethylene				
Trichloroacetic acid	100	62	112	20
Trichloroethanol	—	472	771	129

[A] BEI for benzene calculated for end-of-shift sampling time while methyl chloroform and trichloroethylene calculated at end-of-shift, end-of-workweek

[B] Urinary phenol concentrations adjusted for "background" phenol levels (see text)

ACKNOWLEDGMENTS

The computer simulation and related data analyses were carried out at Colorado State University (CSU). The authors would like to thank Dr. Michael Gargas (McLaren/Hart, ChemRisk Division) for his kind effort in providing the solubility information and Mr. Sean Hays (McLaren/Hart, ChemRisk Division) for his help on mammalian physiology. Finally, the generosity of Dow Chemical Company (Midland, Mich.) in donating the SimuSolv software package is greatly appreciated.

REFERENCES

1. **Fiserova-Bergerova, V.:** Development of biological exposure indices (BEIs) and their implementation. *Appl. Ind. Hyg. 2(2)*:87–92 (1987).
2. **Leung, H.W. and D.J. Paustenbach:** Application of pharmacokinetics to derive biological exposure indexes from threshold limit values. *Am. Ind. Hyg. Assoc. J. 49*:445–450 (1988).
3. **Hermann, E.R. and J.E. Peterson:** Evaluation. In *Fundamentals of Industrial Hygiene*. B.A. Plog, ed. 3rd ed. Washington, DC: National Safety Council, 1988. pp. 387–396.
4. **Perbellini, L., P. Mozzo, D. Olivato, and F. Brugnone:** "Dynamic" biological exposure indexes for n-hexane and 2,5-hexanedione, suggested by a physiologically based pharmacokinetic model. *Am. Ind. Hyg. Assoc. J. 51*:356–362 (1990).
5. **Leung, H.W.:** Use of physiologically based pharmacokinetic models to establish biological exposure indexes. *Am. Ind. Hyg. Assoc. J. 53*:369–374 (1992).
6. **Fiserova-Bergerova, V., J. Vlach, and J.C. Cassady:** Predictable "individual differences" in uptake and excretion of gases and lipid soluble vapours simulation study. *Brit. J. Ind. Med. 37*:42–49 (1980).
7. **Droz, P.O., M.M. Wu, W.G. Cumberland, and M. Berode:** Variability in biological monitoring of solvent exposure: I. Development of a population physiological model. *Brit. J. Ind. Med. 46*:447–460 (1989).
8. **Droz, P.O., M.M. Wu, and W.G. Cumberland:** Variability in biological monitoring of organic solvent exposure: II. Application of a population physiological model. *Brit. J. Ind. Med. 46*:547–558 (1989).
9. **Yang, R.S.H. and M.E. Andersen:** Pharmacokinetics. In *Introduction to Biochemical Toxicology*. E. Hodgson and P.E. Levi, eds. 2nd ed. Norwalk, CT: Appleton and Lange, 1994. pp. 49–73.
10. **El-Masri, H.A., R.S. Thomas, S.A. Benjamin, and R.S.H. Yang:** Physiologically based pharmacokinetic/pharmacodynamic modeling of chemical mixtures and possible applications in risk assessment. *Toxicol.* (1995) [In press]
11. **American Conference of Governmental Industrial Hygienists (ACGIH):** *Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices for 1994–1995*. Cincinnati, OH: ACGIH, 1994.
12. **Ramsey, J.C. and M.E. Andersen:** A physiologically based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol. 73*:159–175 (1984).
13. **Gargas, M.L., M.E. Andersen, and H.J. Clewell:** A physiologically based simulation approach for determining metabolic constants from gas uptake data. *Toxicol. Appl. Pharmacol. 86*:341–352 (1986).
14. **Lindstedt, S.:** "Calculation of allometric scaling factors within and among species." May 25, 1994. [Private communication] Dr. Stan Lindstedt, Design Physiology and Functional Morphology Group, Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011–5640.
15. **Altman, P.L., J.F. Gibson, and C.C. Wang:** Effects of exercise. In *Handbook of Respiration*. D.S. Dittmer and R.M. Grebe, eds. Philadelphia, PA: W.B. Saunders Company, 1958. p. 143.
16. **Chapman, C.B. and R.S. Fraser:** Studies on the effect of exercise on cardiovascular function: cardiac output and mean circulation time. *Circulation 9*:57–62 (1954).
17. **Mitchell, J.H., B.J. Sproule, and C.B. Chapman:** The physiological meaning of the maximal oxygen intake test. *J. Clin. Invest. 37*:538–547 (1958).
18. **McMichael, J. and E.A. Johnston:** Postural changes in cardiac output and respiration in man. *Quart. J. Exp. Physiol. 27*:55–72 (1937).
19. **Holmgren, A., B. Jonsson, and T. Sjostrand:** Circulatory data in normal subjects at rest and during exercise in recumbent position, with special reference to the stroke volume at different work intensities. *Acta Physiol. Scand. 49*:343–363 (1960).
20. **Lundbergh, P. and T. Strandell:** Hepatic wash-out curves of Kr-85 and Xe-133 after retrograde hepatic venous injections in patients with infectious hepatitis and in controls. *Scand. J. Clin. Lab. Invest. 33*:277–285 (1974).
21. **Sherriff, S.B., R.C. Smart, and I. Taylor:** Clinical study of liver blood flow in man measured by Xe-133 clearance after portal vein injection. *Gut 18*:1027–1031 (1977).
22. **Munck, O. and N.A. Lassen:** Bilateral cerebral blood flow and oxygen consumption in man by use of Kr-85. *Circ. Res. 5*:163–168 (1957).
23. **Smith, H.W.:** *The Kidney: Structure and Function in Health and Disease*. New York: Oxford University Press, 1951.
24. **Grimby, G., E. Haggendal, and B. Saltin:** Local xenon 133 clearance from the quadriceps muscle during exercise in man. *J. Appl. Physiol. 22(2)*:305–310 (1967).
25. **Sejrsen, J.:** Blood flow in cutaneous tissue in man studied by washout of radioactive xenon. *Circ. Res. 25*:215–229 (1969).
26. **Lesser, G.T. and S. Deutsch:** Measurement of adipose tissue blood flow and perfusion in man by uptake of Kr-85. *J. Appl. Physiol. 23(5)*:621–630 (1967).
27. **Steiner, E.H.:** Planning and analysis of results of collaborative tests. In *Statistical Manual of the Association of Official Analytical Chemists*. Washington, DC: Association of Official Analytical Chemists, 1975. pp. 66–88.
28. **Vander, A.J., J.H. Sherman, and D.S. Luciano:** *Human Physiology: The Mechanisms of Body Function*. 6th ed. New York: McGraw-Hill, 1994.
29. **Frisancho, A.R.:** New standards of weight and body composition by frame size and height for assessment of nutritional status of adults and the elderly. *Am. J. Clin. Nutr. 40*:808–819 (1984).
30. **Boyd, E.:** Normal variability in weight of the adult human liver and spleen. *Arch. Pathol. 16*:350–372 (1993).
31. **Weibel, E.R.:** *Morphometry of the Human Lung*. Berlin: Springer-Verlag, 1963.
32. **International Commission on Radiological Protection:** *Report of the Task Group on Reference Man*, (No. 23). New York: Pergamon Press, 1975.
33. **Womersley, J., J.V.G.A. Durnin, K. Boddy, and M. Mahaffy:** Influence of muscular development, obesity, and age on the fat-free mass of adults. *J. Appl. Physiol. 41(2)*:223–229 (1976).
34. **Peter, R., R. Bocker, P.H. Beaune, M. Iwasaki, et al.:** Hydroxylation of chlorzoxazone as a specific probe for liver cytochrome P-450IIE1. *Chem. Res. Toxicol. 3*:566–573 (1990).
35. **Reitz, R.H., A.L. Mendrala, and F.P. Guengerich:** *In vitro* metabolism of methylene chloride in human and animal tissues: use in physiologically based pharmacokinetic models. *Toxicol. Appl. Pharmacol. 97*:230–246 (1989).
36. **Datta, D.V., S. Singh, A.K.S. Samanta, S. Saha, et al.:** Determination of bromsulphthalein-glutathione conjugating enzyme activity in needle biopsy specimens of human liver. *Clin. Chem. Acta 47*:133–137 (1973).
37. **Hunter, J., J.D. Maxwell, D.A. Stewart, and R. Williams:** Increased hepatic microsomal enzyme activity from occupational exposure to certain organochlorine pesticides. *Nature 237*:399–401 (1972).
38. **Araki, S.:** Effects of urinary volume on urinary concentrations of lead, δ-aminolaevulinic acid, coproporphyrin, creatinine, and total solutes. *Brit. J. Ind. Med. 37*:50–54 (1980).
39. **Johansson, I. and M. Ingelman-Sundberg:** Benzene metabolism by ethanol-, acetone-, and benzene-inducible cytochrome P-450 IIE1 in rat and rabbit liver microsomes. *Cancer Res. 48*:5387–5390 (1988).
40. **Guengerich, F.P., D.H. Kim, and M. Iwasaki:** Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol. 4*:168–179 (1991).
41. **Raucy, J.L., J.C. Kraner, and J.M. Lasker:** Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit. Rev. Toxicol. 23(1)*:1–20 (1993).
42. **Wrighton, S.A. and J.C. Stevens:** The human hepatic cytochromes p450 involved in drug metabolism. *Crit. Rev. Toxicol. 22(1)*:1–21 (1992).

43. **Andersen, M.E., H.J. Clewell, M.L. Gargas, F.A. Smith, et al.:** Physiologically based pharmacokinetics and the risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol. 87*:185–205 (1987).

44. **van Haaften, A.B. and S.T. Sie:** The measurement of phenol in urine by gas chromatography as a check on benzene exposure. *Am. Ind. Hyg. Assoc. J. 26*:52–58 (1965).

45. **Gargas, M.L., R.J. Burgess, D.E. Voisard, G.H. Cason, et al.:** Partition coefficients of low-molecular-weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol. 98*:87–99 (1989).

46. **Gargas, M.L.:** "Variability in partition coefficients of selected organic compounds." October 21, 1994. [Private communication] Dr. Michael Gargas, McLaren/Hart/ChemRisk, Courtland East Building, 29225 Chagrin Blvd., Cleveland, OH 44122–4629.

47. **Portier, C.J. and N.L. Kaplan:** Variability of safe dose estimates when using complicated models of the carcinogenic process. *Fund. Appl. Toxicol. 13*:533–544 (1989).

48. **Naylor, T., J. Balinty, D. Burdick, and K. Chu:** *Computer Simulation Techniques.* New York: John Wiley & Sons, 1968.

49. **Filliben, J.J.:** The probability plot correlation coefficient test for normality. *Technometrics 17(1)*:111–117 (1975).

50. **American Conference of Governmental Industrial Hygienists (ACGIH):** Documentation of the biological exposure indices. In *Documentation of the Threshold Limit Values and Biological Exposure Indices*, vol. 3, 6th ed. Cincinnati, OH: ACGIH, 1991.

51. **McDougal, J.N., G.W. Jepson, H.J. Clewell, M.G. MacNaughton, et al.:** A physiological pharmacokinetic model for dermal absorption of vapors in the rat. *Toxicol. Appl. Pharmacol. 85*:286–294 (1986).

52. **McDougal, J.N., G.W. Jepson, H.J. Clewell, M.L. Gargas, et al.:** Dermal absorption of organic chemical vapors in rats and humans. *Fund. Appl. Toxicol. 14*:299–308 (1990).

53. **Andersen, M.E. and H.J. Clewell:** "Pharmacokinetic interactions of mixtures." Paper presented at 14th Conference on Environmental Toxicology. (AFAMRL-TR-83–099). Air Force Aerospace Medical Research Laboratory, Wright-Patterson Air Force Base, OH. 1984.

54. **Mumtaz, M.M., I.G. Sipes, H.J. Clewell, and R.S.H. Yang:** Risk assessment of chemical mixtures: biologic and toxicologic issues. *Fund. Appl. Toxicol. 21*:258–269 (1993).

55. **Curtis, L.R., W.L. Williams, and H.M. Mehendale:** Potentiation of the hepatotoxicity of carbon tetrachloride following preexposure to chlordecone (kepone) in the male rat. *Toxicol. Appl. Pharmacol. 51*:283–293 (1979).

56. **Mehendale, H.M.:** Mechanism of the interactive amplification of halomethane hepatotoxicity and lethality by other chemicals. In *Toxicology of Chemical Mixtures: Case Studies, Mechanisms, and Novel Approaches.* R.S.H. Yang, ed. San Diego, CA: Academic Press, 1994. pp. 299–334.