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Variance of Microsomal Protein and Cytochrome P450 2E1 and 3A Forms in Adult Human Liver

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Differences in the pharmacokinetics of xenobiotics among humans makes them differentially susceptible to risk. Differences in enzyme content can mediate pharmacokinetic differences. Microsomal protein is often isolated from liver to characterize enzyme content and activity, but no measures exist to extrapolate these data to the intact liver. Measures were developed from up to 60 samples of adult human liver to characterize the content of microsomal protein and cytochrome P450 (CYP) enzymes. Statistical evaluations are necessary to estimate values far from the mean value. Adult human liver contains 52.9 $\pm$ 1.476 mg microsomal protein per g; 2587 $\pm$ 1.84 pmoles CYP2E1 per g; and 5237 $\pm$ 2.214 pmols CYP3A per g (geometric mean $\pm$ geometric standard deviation). These values are useful for identifying and testing susceptibility as a function of enzyme content when used to extrapolate in vitro rates of chemical metabolism for input to physiologically based pharmacokinetic models which can then be exercised to quantify the effect of variance in enzyme expression on risk-relevant pharmacokinetic outcomes.

Keywords Cytochrome P450, Extrapolation, Human Liver, Pharmacokinetics, Risk Assessment

Human interindividual variance has been recognized for centuries, though at different levels of complexity, and it now poses significant challenges for human health-risk assessment. Pharmacokinetic (PK) and pharmacodynamic (PD) variance are components of the uncertainty factors (UF) used in dose extrapolation by the U.S. Environmental Protection Agency (U.S. EPA). In some instances, risk researchers have replaced default UF values with values derived from actual and toxicologically pertinent data (Murray and Anderson 2001). The recent widespread availability of large banks of human tissue further enables the quantification of more biochemically specific and toxicologically relevant measures of human variance.

Enzymatic variance may have a direct effect on risk-relevant PK outcomes and can be manifested as differences in enzyme content (pmols enzyme/g tissue) or differences in enzymatic specific activity (turnover number; mass of substrate metabolized/min/unit enzyme). The content and activity of enzymes are frequently measured in subcellular fractions (e.g., microsomal protein, MSP) isolated from liver tissue (Fig. 1), so that the extrapolation of results to the intact tissue is complicated. The combination of Michaelis-Menten kinetic parameters (Lipscomb et al. 1997) and estimates of protein content of liver (Lipscomb et al. 1998) have been combined to estimate in vivo PK variance. However, most reports (Ekins et al. 1998; Forrester et al. 1992; Iyer and Sinz 1999; Shimada et al. 1994) do not contain the statistical information necessary to estimate values far from the mean. Rigorous statistical evaluations can be used to quantify the hepatic content of an enzyme (pmols enzyme/g liver tissue) at a given proportion (e.g., 95th percentile) of the population. When linked with (physiologically based pharmacokinetic; PBPK) modeling techniques, this information can allow investigators to quantify the extent to which actual variance in enzyme content contributes to

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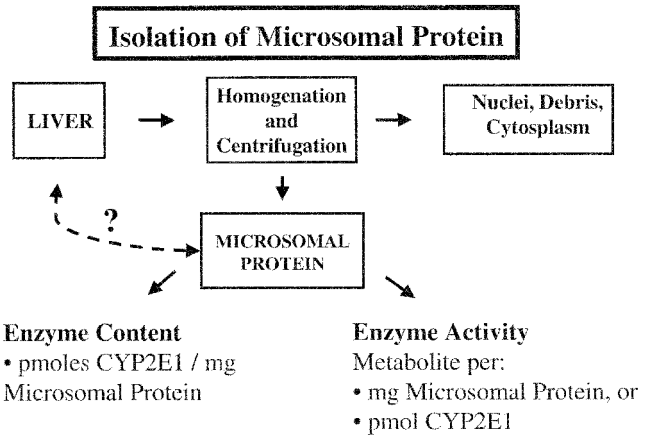

FIG. 1. Isolation of Microsomal Protein. Methods typically employed to isolate microsomal protein from its cellular surroundings preclude the extrapolation of findings of enzyme content and/or enzyme activity to intact liver tissue. Recently, results have demonstrated the utility of extrapolation of in vitro-derived metabolic rates to intact tissues for incorporation in PBPK modeling studies. Extrapolation of enzyme content and enzymatic activity is enabled with knowledge of the amount of liver homogenized, the volume of the homogenate, and the protein content of the homogenate.

toxicologically relevant PK measures (Lipscomb and Kedderis 2002). To determine the statistical bounds of a defined segment of the population, we had previously collected data that provide an adequate statistical measure of the human interindividual variance of CYP2E1 and other forms in hepatic MSP from 40 adult organ donors (Snawder and Lipscomb 2000). Those data provided the necessary information on (CYP) forms in MSP, but no data existed on the distribution of CYP forms in intact liver.

Since that time, we have quantified the expression of CYP2E1 and 3A forms in hepatic MSP from an additional 20 human organ donors beyond the 40 evaluated by Snawder and Lipscomb (2000). We have determined and herein present the measures and variances in the hepatic content of MSP, total protein, CYP2E1, and CYP3A forms in samples of liver tissues from 20 adult human organ donors. Further, this report combines those data with previously published data on 40 other human hepatic MSP samples and describes the statistical distribution of CYP2E1 and CYP3A forms in intact human liver. This article (1) communicates those data, (2) discusses necessary analytic conditions, (3) identifies other situations to which the data may be applicable, and (4) illustrates their potential employment in quantifying human interindividual metabolic and PK variance for potential inclusion in safety and risk assessments.

MATERIALS AND METHODS

Samples

Samples of human donor liver tissue were obtained from Tissue Transformation Technologies (Exton, PA). Donor information is presented in Table 1. Tissues were homogenized, and MSP was isolated according to the method of Guengerich (1989). Tissue homogenates were centrifuged 100*g* to sediment nuclei and cellular debris. The protein content of homogenates and resulting microsomal fractions was determined in duplicate by using the Bradford method (1976). Homogenates and microsomes were stored at -70°C until analysis.

Quantification of CYP Proteins

The quantification of CYP proteins via enzyme-linked immunosorbent assay (ELISA) was conducted on both homogenate and microsomal protein as previously described (Snawder and Lipscomb 2000). For each donor tissue, duplicate samples of post-100*g* homogenates and MSP were analyzed for CYP2E1 and CYP3A content. The CYP content of intact liver was calculated for each CYP form by multiplying the CYP content of homogenate protein (i.e., pmols CYP2E1/mg homogenate protein) by the yield of homogenate protein (e.g., mg homogenate protein/g liver tissue).

Estimation of Proteins in Intact Liver

The total amount of protein in intact liver was empirically determined on the basis of the protein content of the post-100*g* liver supernatant by accounting for the wet weight of the tissue, the volume of resulting tissue homogenate, the protein content of the homogenate, and the microsomal protein suspension. The hepatic content of CYP forms (pmols CYP/g liver) were estimated by statistically combining data sets as described later.

The amount of MSP per g liver was estimated according to Equation (1). Results for CYP2E1 and CYP3A were averaged within a given organ donor ($n = 20$).

$$[((\text{pmol CYP2E1/g tissue})/(\text{pmol CYP2E1/mg MSP})) + ((\text{pmol CYP3A/g tissue})/(\text{pmol CYP3A/mg MSP}))]/2 = (\text{mg MSP/g liver}) \quad [1]$$

Statistical Analysis

Probability distributions were fitted by the SAS 8.0 Analyst routine to data describing the following variables (with variable name): mg homogenate protein/g liver (PRO_{Liv}); pmol CYP2E1/mg homogenate protein ($CYP2_{Pro}$); pmol CYP3A/mg homogenate protein ($CYP3_{Pro}$); pmol CYP2E1/mg MSP ($CYP2_{MSP}$); pmol CYP3A/mg MSP ($CYP2_{MSP}$); pmol CYP2E1/g liver ($CYP2_{Liv}$); pmol CYP3A/g liver ($CYP3_{Liv}$); mg MSP/g liver (MSP_{Liv}); nmol P450/mg MSP ($P450_{MSP}$). Two sets of data were available and each was analyzed separately: a set of $n = 60$ samples, for which only three laboratory measurements were available ($CYP2_{MSP}$, $CYP3_{MSP}$, and $P450_{MSP}$); and a subset of the 60 samples $n = 20$, for which several additional laboratory measurements were available (PRO_{Liv}, $CYP2_{Pro}$, and $CYP3_{Pro}$). The additional variables ($CYP2_{Liv}$, $CYP3_{Liv}$, and MSP_{Liv}) were calculated from the measurement data. For all sets of observations, four distributions—normal, lognormal, exponential, and Weibull—were evaluated using standard statistical tests of goodness-of-fit (Kolmogorov-Smirnoff, Cramer-von Mises, and Anderson-Darling) and a visual examination of quantile-quantile plots. The null hypothesis was that the distribution fit the data well, with a rejection of the null at $p \leq .10$. All analyses were performed using SAS software

TABLE 1
Donor demographics

Age/Sex[a]	Donor	HHM[b]	COD[c]	Height/weight	BMI[d]	Alcohol consumption	Cigarette smoking
54 CM	628951	234	SAH	5′7″/135 lb.	21.2	1 Quart/day × 30–40 years	2 ppd × 30–40 year
42 CF	625961	244	Anoxia	6′6″/209 lb.	24.2	NR	1.5 ppd/25 years
45 CM	730961	226	SAH	6′1″/200 lb.	26.4	12 Beers/week	5–6 ppd
77 CF	513941	179	SAH	5′2″/186 lb.	34.1	NR	NR
54 HF	1103911	134	SAH	5′5″/158 lb.	26.3	3 Drinks/day	NR
42 CF	317941	237	SAH	5′4″/482 lb.	82.9	Unknown	1 ppd × 20 years
45 BM	218962	238	SAH	5′10″/257 lb.	37.0	Social, quit 7 years	Quit 15 years
61 CM	325941	150	SAH	6′/197 lb.	26.8	Quit 11 years	Quit 11 years
31 CM	825941	202	GSW-H	5′10″/160 lb.	23.0	Beer	1.5 ppd × 5–6 years
63 BM	711953	243	CHI	5′11″/198 lb.	27.7	1 Gallon gin/day	1 ppd × 20 years[e]
36 CF	102921	68	GSW-H	5′8″/242 lb.	36.9	NR	NR
32 CM	305951	189	CHI	5′10″/190 lb.	27.3	Social	1 ppd/10 years
50 HF	310921	131	SAH	5′5″/250 lb.	41.7	Unknown	NR
28 CM	320951	129	SAH	5′5″/170 lb.	28.3	Daily	Rare
32 CM	416931	112	GSW-H	5′8″/140 lb.	21.3	Social	1 ppd
46 CM	518931	156	CHI	6′2″/220 lb.	28.3	NR	NR
19 HM	823941	133	SIGSW	NR/NR	Unknown	NR	NR
48 CF	926931	180	CVA	5′3″/185 lb.	32.8	NR	Quit 1 year
38 CF	929942	239	Anoxia	5′6″/77 lb.	12.5	NR	NR
41 CF	1101931	138	GSW	5′8″/222 lb.	33.2	Unknown	2 ppd × 10 years

[a] Samples are identified by age in years, ethnic background, and sex; B, black; C, Caucasian; H, Hispanic; F, female; M, male. Cigarette smoking is reported in packs per day (ppd) when quantified. Ethanol consumption was subjectively reported. NR, a negative response, was given for cigarettes or ethanol.
[b] Human Hepatic Microsome Sample Number, Tissue Transformation Technologies, Exton, PA.
[c] CHI, closed head injury; COD, cause of death; CVA, cerebrovascular accident, GSW, gunshot wound; GSW-H, GSW-head; SAH, subanachnoid hemorrhage; SIGSW, self-inflicted GSW.
[d] BMI, body mass index, calculated as kg/(m^3).
[e] Quit 10 years ago.

(SAS Statistical Institute, Cary, NC). Each of the sets of observations was adequately fit by the lognormal distribution, the parameters of which are the mean (μ) and standard deviations of the logarithms of the observations. All goodness-of-fit *p* values were greater than .15. As a convenience, the log-normal parameter is represented by the geometric mean ($GM = e^{\mu}$) and the geometric standard deviation ($GSD = e^{s}$), respectively, in this paper. Equation (2) demonstrates the method used to estimate the distribution of the CYP2E1 and 3A forms in the intact liver, where the distribution for $CYP2_{MSP}$ or $CYP3_{MSP}$ was multiplied by the distribution for MSP_{Liv}. The values at the 5th (X_{05}) and 95th (X_{95}) percentiles were calculated by applying Equations (3), (4), and (5), respectively.

$$\text{L norm}\,(\mu = \mu_1 + \mu_2,\ \text{s} = \text{sq root}\left(s_1^2 + s_2^2\right) \qquad [3]$$

where μ_i = mean of logs of observations; s_i = standard deviation of logs of observations; 1 = data set 1 ($CYP2_{MSP}$ or $CYP3_{MSP}$); and 2 = data set 2 (MSP_{Liv}).

$$X_{05} = e^{[\mu - 1.645 * s]} \qquad [4]$$

$$X_{95} = e^{[\mu + 1.645 * s]} \qquad [5]$$

RESULTS

In no instance was the difference between replicates of total protein (Bradford) or CYP proteins (ELISA) more than 10%. The intact tissue content of homogenate protein, MSP, CYP2E1, and CYP3A forms are presented in Table 2. These results quantify the content of total protein and of MSP, CYP2E1, and CYP3A proteins in adult human liver and are presented as the arithmetic mean and standard deviation (SD) and the more technically correct geometric mean and geometric standard deviation. MSP accounts for 43.2 ± 15.3% (mean ± SD) of total protein (range 20–89%). The lognormal distribution fit the data best for most variables; the normal distribution was either a better or an equivalent fit for $CYP2_{Pro}$, $CYP3_{Pro}$, and $CYP3_{Liv}$. Neither the Weibull nor the exponential distribution was selected as the best fit for any parameter. As lognormal distributions are generally adequate for these data, the analytical solution for multiplicative lognormal distributions (Equation (2)) can be used as a computational convenience when the data sets are independent. The GM and GSD values in Table 2 were used as the lognormal parameters for the relevant variables to estimate the 90% confidence interval of the hepatic CYP content of the adult

TABLE 2
Total protein and cytochrome P450 in liver tissue from 20 adult organ donors

			pmols CYP2E1 per			pmols CYP3A per		
Donor	mg Total protein/g liver (PRO_{Liv})	mg MSP/g liver (MSP_{Liv})	mg Homog protein ($CYP2_{Pro}$)	g Liver ($CYP2_{Liv}$)	mg MSP ($CYP2_{MSP}$)	mg Homog protein ($CYP3_{Pro}$)	g Liver ($CYP3_{Liv}$)	mg MSP ($CYP3_{MSP}$)
628951	134	27.0	16.1	2147	86	26.6	3552	123
625961	101	34.4	25.6	2591	100	31.7	3209	75
730961	137	34.3	17.9	2439	83	34.6	4721	120
513941	100	41.5	15.2	1510	23	23.7	2363	137
1103911	113	37.2	10.9	1229	34	24.7	2792	73
317941	151	54.7	12.2	1836	36	40.1	6060	103
218962	154	57.1	25.0	3852	77	30.0	4615	72
325941	148	66.1	12.4	1822	46	37.0	5458	59
825941	115	38.2	21.5	2463	69	53.6	6148	151
711953	137	71.8	20.9	2858	59	45.0	6161	65
102921	181	90.2	24.6	4439	54	39.2	7079	72
305951	180	90.1	27.8	5007	64	41.4	7448	73
310921	126	55.4	21.4	2692	68	36.7	4627	65
320951	124	49.1	21.9	2712	53	44.5	5505	117
416931	122	108.3	22.3	2709	46	45.4	5522	35
518931	137	47.4	24.4	3352	66	34.0	4670	106
823941	152	45.0	16.3	2464	41	24.0	3638	122
926931	130	85.1	24.1	3143	24	40.9	5335	136
929942	69	34.7	25.2	1724	42	33.2	2275	80
1101931	126	70.4	14.0	1752	41	32.8	4119	42
Mean (SD)	132 (26.3)	56.9 (22.7)	20.0 (5.18)	53.9 (23.4)	55.6 (21.0)	36.0 (8.02)	116.5 (65.9)	91.3 (33.5)
GM (GSD)	129 (1.24)	52.9 (1.48)	19.3 (1.33)	2482 (1.43)	51.7 (1.50)	35.1 (1.26)	4525 (1.41)	85.1 (1.49)

human organ-donor population (generalized to the adult human population).

Dividing the arithmetic mean values of $CYP2_{Liv}$ and $CYP3_{Liv}$ by the arithmetic mean values of $CYP2_{MSP}$ and $CYP3_{MSP}$ and using available published information for $CYP2_{MSP}$ and $CYP3_{MSP}$ (Shimada et al. 1994) indicate that the average MSP content of human liver (MSP_{Liv}) is approximately 50 mg MSP per g intact tissue. This compares favorably with MSP_{Liv} values calculated independently from the same sample set and presented in Table 2 and with values calculated exclusively from data describing the CYP2E1 content of liver homogenate protein and MSP (GM 48.03; GSD 1.494 mg MSP/g liver). However, because of a slight inverse correlation between pmols CYP2E1/mg MSP and mg MSP/g liver, as estimated from ELISA data describing the content of CYP2E1 in homogenate protein and in MSP, combining distribution parameters derived for MSP_{Liv} and $CYP2_{MSP}$ overestimates the variance of CYP2E1 in intact tissue. The variance in the CYP2E1 content of intact human liver may be best described by the data describing the CYP2E1 content of the homogenate protein (2482 GM; 1.43 GSD pmols CYP2E1/g liver, $n = 20$; see Table 2). Results derived exclusively from ELISA data describing the CYP3A content of homogenate protein and MSP ($n = 20$) indicate that MSP is present in intact liver at a concentration of 61.7 ($\pm$35.4, arithmetic mean $\pm$ SD; variance 1252); the minimum value was 17.3, and the maximum value was 158 mg MSP/g liver. The geometric mean for the sample set was 53.23 mg MSP/g liver, and the geometric SD was 1.750. From these parameters and from values derived exclusively from data demonstrating CYP3A content, the values at the 5th and 95th percentiles were determined to be 21.19 and 133.6 mg MSP/g liver, respectively.

No correlation was observed between the values for mg MSP/g liver and the CYP3A content of MSP. However, the arithmetic and geometric means for the MSP content of intact liver, whether derived from ELISA data describing CYP3A forms or describing CYP2E1 forms, were quite similar. Thus, values for the combined data set are shown in Table 3 (MSP_{Liv}). Based on these results, liver tissues from adult human organ donors contains 52.9 ± 1.476 (GM $\pm$ GSD) mg MSP per g. Spectrally determined CYP (nmol/mg MSP) was lognormally distributed with a GM of 0.34 and GSD of 1.6 ($n = 60$). The content of CYP forms is rarely determined in intact tissue. Therefore, we undertook to establish reference "library" distributions for the $CYP2_{MSP}$ and $CYP3_{MSP}$ variables using the larger data set of 60 observations.

TABLE 3
Log-normal distributions for microsomal protein, CYP2E1 and CYP3A content of human liver: comparison of fit to measured values ($n = 20$) and derived fit using extended data set ($n = 60$)

				$CYP2_{Liv}$[d]		$CYP3_{Liv}$[e]	
	MSP_{Liv}[a]	$CYP2_{MSP}$[b]	$CYP3_{MSP}$[c]	Measured[f]	Derived	Measured[f]	Derived
GM	52.9	48.9	99	2482	2587[g]	4525	5237[g]
GSD	1.476	1.6	2	1.43	1.84[g]	1.41	2.214[g]
Range	27–108	11–130	35–312	1229–5007	—	2363–7448	—
5th Percentile[h]	27.9	22.5	31.6	1378	949	2571	1416
95th Percentile[i]	100	106	310	4470	7053	7963	19,364

[a] mg microsomal protein/g liver; from Table 2 ($n = 20$).
[b] pmols CYP2E1/mg microsomal protein; from extended data set ($n = 60$).
[c] pmols CYP3A/mg microsomal protein; from extended data set ($n = 60$).
[d] pmols CYP2E1/g liver.
[e] pmols CYP3A/g liver.
[f] From Table 2 ($n = 20$).
[g] From Equation (4).
[h] From Equation (5).
[i] From Equation (6).

The library ($n = 60$) distributions for $CYP2_{MSP}$ and $CYP3_{MSP}$ can be used in Equation (2) to predict hepatic CYP enzyme content ($CYP2_{Liv}$, $CYP3_{Liv}$) when combined with MSP_{Liv} distribution. However, this will result in an overestimation of variance in CYP2E1 content, given the dependence of data, as previously noted. For that reason, the most reliable estimates of variance in the hepatic content of CYP2E1 are those based on the measurement of CYP2E1 content in the homogenate protein. Table 3 presents the results of the distributional analysis of CYP enzyme content, comparing the measured values for $CYP3_{Liv}$ (in 20 samples) with the predicted ("derived") values based on Equation (4) and using the library ($n = 60$) distributions for $CYP3_{MSP}$ combined with MSP_{LIV}. It is immediately evident that the variance of the empirically measured distributions (sample set of $n = 20$) is substantially smaller than that of the derived values (sample set n up to 60; Table 3 columns labeled *Measured* and *Derived*), yielding somewhat "conservative" estimates of values at the extreme percentiles: both the 5th and 95th percentile values are well outside the range of the observations. The differences are most likely a result of a moderate correlation between MSP_{Liv} and the CYP_{MSP} variables (trimmed correlations $= -0.31$ to -0.50) (Gnanadesikan and Kettenring 1972). Correlations in the input variables cannot be handled directly in Equation (4), but could be accounted for by use of a Monte Carlo approach if the analytic approximation were deemed inadequate for the application.

DISCUSSION

The successful and accurate identification of the bounds and the distribution of sensitivity in the population is necessary so as to ensure that protective exposure limits are established. PK variance among humans has been investigated as a risk-modifying factor for CYP2E1 substrates, including toluene and chloroform (Allen et al. 1996; Pierce et al. 1996). Often, interindividual variance among humans in xenobiotic metabolism alters the pharmacokinetics, thus mediating differences in susceptibility to injury. A well-known example is the polymorphic expression of CYP2D6 (Tanaka 1999). Some studies have demonstrated the value of biochemical assessment prior to the administration of therapeutic agents whose metabolizing and elimination (PK) are dependent on this form (Chou et al. 2000; Guzey et al. 2000). Nonpolymorphically expressed enzymes can be influenced by lifestyle, genetics, and environmental factors (de la Maza et al. 2000). The overexpression of CYP2E1 in individuals who are exposed to alcohol or other known inducers and the increased expression of CYP1A forms in those who smoke cigarettes are some examples. Thus, the examination of the distribution in the population of xenobiotic metabolizing enzymes whose activities may directly influence risk can yield valuable information about the magnitude of sensitivity among the segment of the population whose enzyme expression is divergent from mean values.

To refine estimates of risk, the injury from the xenobiotic risk agent must be tied to PK outcomes, which are influenced by differences in metabolism (pharmacokinetics, clearance). Quantitative estimates of metabolism should be tied to units of MSP and, more specifically, to a CYP (or other enzyme) form for which an adequate statistical analysis of human expression and variance in expression has been determined. This report demonstrates a broadly based and sound quantification of the expression of and variance in CYP2E1 and CYP3A forms in adult organ donors. Furthermore, the methods presented here allow for a probabilistic expression of interindividual variance in xenobiotic metabolism that can be of use in risk-assessment models.

The MSP content in liver has been previously used in the extrapolation of in vitro rates of metabolism of perchloroethylene (Reitz et al. 1996) and trichloroethylene (Lipscomb et al. 1998) for inclusion in PBPK models. In addition, MSP content has been used to estimate the intrinsic clearance of several therapeutic agents (Carlile 1999). The present data set describing the statistical measures of the expression of MSP (see Table 3) in tissue from 20 adult human organ donors represents a significant advance over data previously available. These data on MSP_{LIV} can be used to extrapolate rates of metabolism dependent on enzymes other than CYP forms that are also contained in the MSP such as glucuronyl transferases. In addition, our data describing the distribution of CYP2E1 and CYP3A forms in intact liver tissue can be employed to extrapolate in vitro–derived metabolic rates (for example, Kanamitsu et al. 2000) for chemicals that are metabolized by either form. These include a great many environmental contaminants, therapeutic agents, and occupation-based chemicals. CYP3A4 is believed to be responsible for the metabolism (clearance) of more than 50% of therapeutic agents, while CYP2E1 metabolizes a great many volatile organic solvents of low molecular weight. Data describing the human interindividual variance of these forms may be linked with PBPK models to determine the extent to which real, not hypothesized, differences in human metabolic capacity can influence risk-relevant PK outcomes. This approach may be useful in performing assessments of the anticipated PK of potential therapeutics during the preclinical phase or of those agents whose demonstrated toxicity precludes the generation of data from human studies conducted in vivo. In an alternative approach to PBPK modeling, differences in metabolic clearance can be compared to rates of delivery so as to estimate whether differences in metabolism may mediate differences in clearance. Differences in the expression of these enzymes are unlikely to mediate differences in their metabolic clearance of agents that are delivered in blood to the liver at rates (dependent on dose, solubility in blood, and hepatic blood flow rate) at or below the lower boundary of their metabolizing. However, the expression and activity of CYP forms in the intestine should be considered in technical estimates of the impact of enzyme expression on metabolic clearance.

This report presents data useful in the evaluation of human interindividual metabolic and PK variance. These results can lead to the quantitative determination of the impact of variance in enzyme expression on PK outcomes. For PBPK models addressing the metabolizing and elimination of compounds metabolized by either CYP2E1 forms (styrene, perchloroethylene, trichloroethylene, chloroform, etc.) or CYP3A forms (erythromycin, triazolam, cyclosporin), these data can be used to generate population bounds for inclusion in sensitivity analyses of the impact of model parameters. This approach surpasses the generic examination of the impact of a hypothetical variance of 10%, as is the common practice. This advance will allow for a more scientifically (biologically) based examination of the impact of metabolic variance, PK variance, and susceptibility, as mediated by differences in the CYP enzyme and MSP content of human liver.

REFERENCES

Allen, B. C., Covington, T. R., and Clewell, H. J. 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. *Toxicology* 111:289–303.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. 72:248–254.

Carlile, D. J., Hakooz, N., Bayliss, M. K., and Houston, J. B. 1999. Microsomal prediction of in vivo clearance of CYP2C9 substrates in humans. *Br. J. Clin. Pharmacol*. 47:625–635.

Chou, W. H., Yan, F. X., de Leon, J., Barnhill, J., Rogers, T., Cronin, M., Pho, M., Xiao, V., Ryder, T. B., Liu, W. W., Teiling, C., and Wedlund, P. J. 2000. Extension of a pilot study: impact from the cytochrome P450 2D6 polymorphism on outcome and costs associated with severe mental illness. *J. Clin. Psychopharmacol*. 20:246–251.

de la Maza, M. P., Hirsch, S., Petermann, M., Suazo, M., Ugarte, G., and Bunout, D. 2000. Changes in microsomal activity in alcoholism and obesity. *Alcohol Clin. Exp. Res*. 24:605–610.

Ekins, S., Vandenbranden, M., Ring, B., Gillespie, J. S., Yang, T. J., Gelboin, H. V., and Wrighton, S. A. 1998. Further characterization of the expression in liver and catalytic activity of CYP2B6. *J. Pharmacol. Exp. Ther*. 286:1253–1259.

Forrester, L. M., Henderson, C. J., Glancey, M. J., Back, D. J., Park, B. K., Ball, S. E., Kitteringham, N. R., McLaren, A. W., Miles, J. S., Skett, P., and Wolf, C. R. 1992. Relative expression of cytochrome P450 isoenzymes in human liver and association with the metabolism of drugs and xenobiotics. *Biochem. J*. 281:359–368.

Gnanadesikan, R., and Kettenring, J. R. 1972. Robust estimates, residuals, and outlier detection with multiresponse data. *Biometrics* 28:81–124.

Guengerich, F. P. 1989. Analysis and characterization of enzymes. In *Principles and Methods of Toxicology*, ed. A. W. Hayes, 777–814. New York: Raven Press.

Guzey, C., Aamo, T., and Spigset, O. 2000. Risperidone metabolism and the impact of being a cytochrome P450 2D6 ultrarapid metabolizer. *J. Clin. Psychiatry* 61:600–601.

Iyer, K. R., and Sinz, M. W. 1999. Characterization of phase I and phase II hepatic drug metabolism activities in a panel of human liver preparations. *Chem. Biol. Interact*. 118:151–169.

Kanamitsu, S., Ito, K., Green, C. E., Tyson, C. A., Shimada, N., and Sugiyama, Y. 2000. Prediction of in vivo interaction between triazolam and erythromycin based on in vitro studies using human liver microsomes and recombinant human CYP3A4. *Pharm. Res*. 17:419–426.

Lipscomb, J. C., Fisher, J. W., Confer, P. D., and Byczkowski, J. Z. 1998. In vitro to in vivo extrapolation for trichloroethylene metabolism in humans. *Toxicol. Appl. Pharmacol*. 152:376–387.

Lipscomb, J. C., Garrett, C. M., and Snawder, J. E. 1997. Cytochrome P450–dependent metabolism of trichloroethylene: Interindividual differences in humans. *Toxicol. Appl. Pharmacol*. 142:311–318.

Lipscomb, J. C., and Kedderis, G. L. 2002. Incorporating human interindividual biotransformation variance in health risk assessment. *Sci. Total Environ*. 288:13–21.

Murray, F. J., and Andersen, M. E. 2001. Data-derived uncertainty factors: boric acid (BA) as a case study. *Hum. Ecol. Risk Assess*. 7:125–138.

Pierce, C. H., Dills, R. L., Morgan, M. S., Nothstein, G. L., Shen, D. D., and Kalman, D. A. 1996. Interindividual differences in 2H8-toluene toxicokinetics assessed by semiempirical physiologically based model. *Toxicol. Appl. Pharmacol*. 139:49–61.

Reitz, R. H., Gargas, M. L., Mendrala, A. L., and Schumann, A. M. 1996. In vivo and in vitro studies of perchloroethylene metabolism for physiologically

based pharmacokinetic modeling in rats, mice and humans. *Toxicol. Appl. Pharmacol*. 136:289–306.

Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., and Guengerich, F. P. 1994. Interindividual variations in human liver cytochrome P450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther*. 270:414–423.

Snawder, J. E., and Lipscomb, J. C. 2000. Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. *Regul. Toxicol. Pharmacol*. 32:200–209.

Tanaka, E. 1999. Update: genetic polymorphism of drug metabolizing enzymes in humans. *J. Clin. Pharm. Ther*. 24:323–329.