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# Interindividual Variance of Cytochrome P450 Forms in Human Hepatic Microsomes: Correlation of Individual Forms with Xenobiotic Metabolism and Implications in Risk Assessment

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**Differences in biotransformation activities may alter the bioavailability or efficacy of drugs, provide protection from certain xenobiotic and environmental agents, or increase toxicity of others. Cytochrome P450 (CYP450) enzymes are responsible for the majority of oxidation reactions of drugs and other xenobiotics and differences in their expression may directly produce interindividual differences in susceptibility to compounds whose toxicity is modulated by these enzymes. To rapidly quantify CYP450 forms in human hepatic microsomes, we developed, and applied, an ELISA to 40 samples of microsomes from adult human organ donors. The procedure was reliable and the results were reproducible within normal limits. Protein content for CYP1A, CYP2E1, and CYP3A positively correlated with suitable marker activities. CYP1A, CYP2B, CYP2C6, CYP2C11, CYP2E1, and CYP3A protein content demonstrated 36-, 13-, 11-, 2-, 12-, and 22-fold differences between the highest and lowest samples and the values were normally distributed. Of the forms examined, CYP3A was expressed in the highest amount and it was the only form whose content was correlated with total CYP450 content. Content of other forms was independent of total CYP450. We further determined the contribution of specific forms to the biotransformation of trichloroethylene as a model substrate. CYP2E1 was strongly correlated with chloral hydrate formation from trichloroethylene; CYP2B displayed the strongest correlation with trichloroethanol formation. These data describing the expression and distribution of these forms in human microsomes can be used to extrapolate *in vitro* derived metabolic rates for toxicologically important reactions, when form selectivity and specific activity are known. This approach may be applied to refine estimates of human interindividual differences in susceptibility for application in human health risk assessment.**

***Key Words:*** **risk assessment; uncertainty factors; cytochrome P450; human variance; microsomes; ELISA; metabolism.**

## INTRODUCTION

The U.S. Environmental Protection Agency's (EPA) current system for managing noncarcinogenic risks posed by chemicals requires that human exposures not exceed a reference dose (RfD) (U.S. EPA, 1988). U.S. EPA defines the RfD as "an estimate (with uncertainty spanning perhaps an order of magnitude) of daily exposure to the human population that is likely to be without appreciable risk of deleterious effects during a lifetime"; it is generally interpreted to be a concentration of a chemical that is "safe" to ingest on a daily basis over a lifetime (Barnes and Dourson, 1988). The RfD is derived from a critical effect level: either a no-observed-adverse-effect level (NOAEL) or a lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study. The RfD is calculated by dividing the critical effect level by the product of one or more uncertainty factors and a modifying factor (Table 1). The uncertainty factors are applied because the RfD methodology requires an adjustment of NOAEL or LOAEL values (whether from experimental animal or human studies) to account for pharmacokinetic and pharmacodynamic differences within and between species as well as other sources of uncertainty in a data set (U.S. EPA, 1988).

In this paper we assume that *in vitro* human pharmacokinetic and pharmacodynamic data can inform the choice of $UF_H$, the variation in susceptibility among the human population. Swartout *et al.* (1998) describe this factor as accounting for the uncertainty in estimat-

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**TABLE 1**
**Uncertainty Factors and Associated Extrapolations Across Endpoints**[a]

| Uncertainty factor | Estimated endpoint | Measured endpoint |
|---|---|---|
| Interindividual ($UF_H$) | NOAEL in sensitive subpopulation | NOAEL in general population |
| Interspecies ($UF_A$) | NOAEL in typically healthy human population | NOAEL in animal test species |
| Subchronic ($UF_S$) | NOAEL in a chronic study | NOAEL in a subchronic study |
| LOAEL ($UF_L$) | NOAEL in a study | LOAEL in a study |
| Database ($UF_D$) | The lowest NOAEL observed in a set of toxicologic studies | NOAEL in a chronic study |

[a] Adapted from Swartout *et al.* (1998).

ing the NOAEL in a sensitive subpopulation of humans given the NOAEL in a human subpopulation whose chemical susceptibility is not assumed to include that of the sensitive subpopulation (see Table 1). While conceptually the lower bound estimate for this factor is 1 (meaning that the NOAEL is derived from a study of the most susceptible human population or that there is no difference anticipated in susceptibility across the human population), the range of interindividual susceptibility is uncertain for most compounds.

This conceptual approach for quantifying $UF_H$ assumes that an understanding of the human heterogeneity in key metabolic pathways sufficiently describes interindividual susceptibility. Metabolic heterogeneity may arise through genetic or physiologic differences in the population. The identification and development of data which define the variance in metabolic processes that activate or detoxify environmental toxicants as well as those that eliminate environmental toxicants is critical to implementation of this type of approach. Assessment of the distribution of target tissue susceptibility [a pharmacodynamic component] across the human population as well as other elements of $UF_H$ is outside the scope of this paper.

The CYP450-dependent monooxygenase system is responsible for the majority of oxidation reactions of drugs and other xenobiotics and thus critically impacts the pharmacokinetics of these chemicals. Variations in these biotransformation activities may alter the bioavailability or efficacy of drugs, provide protection from certain xenobiotic agents, or increase toxicity of others. Interspecies and interindividual variation may result from genetic or environmental influence (Okey, 1990). Physiological and pathological conditions such as age, sex, fasting, hepatic damage, diabetes, and obesity have all been demonstrated to alter biotransformation activities in humans and experimental animals (Kato and Yamazoe, 1992; Barnett *et al.,* 1993). The extent to which these forms differ in expression and activity among humans has a marked influence on the pharmacokinetics and toxicological (pharmacodynamic) effects of xenobiotics. Of the many families of cytochrome P450 (CYP450), the CYP1, CYP2, and CYP3 families appear to be the most important in the metabolism of most drugs and xenobiotics (Kadlubar and Guengerich, 1992).

Numerous means have been used to identify the CYP450 forms involved in the metabolism of various agents. Purified and reconstituted human CYP450 forms, heterologous cells that express functional human CYP450 forms, metabolic competition assays, and immunoinhibition studies have proven invaluable in identifying the extent to which different forms contribute to the metabolism of various substrates (Snawder *et al.,* 1994). Though these are invaluable tools in determining the forms responsible for metabolic activity toward a particular substrate, they provide no information on the extent to which these forms and their dependent activities may vary among the general population. While *in vitro* metabolism studies may not be as ideal as *in vivo* studies, in the case of human metabolism to various agents, *in vitro* studies are often the only option. With the increasing availability of organ donor hepatic microsomes, large banks of samples may be obtained representing a cross-section of the population. Individual microsomal samples may be "phenotyped" in regard to CYP450-dependent activities as well as content of specific forms. Batteries of these well-characterized microsome samples can be employed in studies to determine the fate of test chemicals and the extent of variation of hepatic metabolism which may be reasonably anticipated to occur *in vivo* for compounds whose metabolism is not limited by delivery to the liver.

Because of the industrial and environmental significance of trichloroethylene (TCE), the modulation of TCE's toxicity by CYP-dependent metabolism and the EPA's ongoing reassessment of TCE's human health risk, we previously employed a battery of 23 human microsome samples to assess differences in rates of CYP-dependent TCE metabolism (Lipscomb *et al.,* 1997). The results of this study revealed a significant correlation of TCE metabolism with chlorzoxazone metabolism, a marker for CYP2E1-dependent activities in the human, and an approximate sevenfold difference in $V_{max}$ values toward TCE among the sample set evaluated. In a subsequent study, the CYP-dependent metabolic rates for TCE derived from these studies and with multiple isolated human hepatocyte samples were extrapolated to rates reflective of the intact human liver. These extrapolated rates were then integrated into a physiologically based pharmacokinetic (PBPK) model for the human. The results of this PBPK model demonstrated the interindividual variance of pharmacokinetic parameters

which had their basis in differences in rates of metabolism (Lipscomb *et al.*, 1998a). The combination of these techniques demonstrates the successful and relevant application of data derived from *in vitro* studies addressing the basis of human interindividual variance in chemical metabolism.

Earlier investigations (Shimada *et al.*, 1994) have employed SDS–PAGE to quantify CYP forms in multiple human hepatic microsome samples and demonstrated a high level of correlation between form-specific CYP expression and form-selective activities. This characterization of human variance did not explicitly tie the distribution of CYP protein expression with direct measures of metabolism of environmentally important compounds. Because of the technical complications of electrophoresis and the previous success with application of the enzyme-linked immunosorbent assay (ELISA) technique to quantify differences in the expression of CYP2E1 in mice (Roe *et al.*, 1999), we have assessed the technique's ability to discern human interindividual variations of form-selective CYP expression. In the present study, we have determined the content of multiple CYP forms from the 1A, 2B, 2C, 2E, and 3A families in up to 40 samples of human hepatic microsomes. The antibodies employed were those commercially available and directed against CYP forms from rats. The protein content of these CYP forms were individually correlated with metabolic activities toward substrates deemed specific to the forms as expressed in human microsomes. We have further examined the microsome-mediated conversion of TCE to chloral hydrate and trichloroethanol and quantified the extent to which TCE bioconversion depends upon individual CYP forms across multiple human samples. For compounds whose toxic consequences are modulated by metabolism by identified CYP forms, our data describing the human interindividual variance of the expression of the relevant form may be used to estimate the degree of human interindividual variance of its metabolism. These data represent a significant advance in quantifying human interindividual variance and may be directly applicable in estimating $UF_H$ for specific toxicants.

## MATERIALS AND METHODS

*Chemicals.* All chemicals were at least reagent grade and were obtained from Sigma (St. Louis, MO) or Aldrich (Milwaukee, WI) unless otherwise noted. Glucose 6-phosphate and NADP+ were obtained from Boehringer–Mannhein (Indianapolis, IN), and ketoconazole was purchased from Janssen Biotech (Olen, Belgium).

*Microsome preparation and metabolism of TCE.* Human microsomes obtained from a commercial supplier (International Institute for the Advancement of Medicine, Exton, PA) were prepared via the method of Guengerich (1989). Microsomal protein content was determined by the BCA method with bovine serum albumin as a standard. Total CYP450 was determined using the differential spectrophotometric method of Omura and Sato (1964). Some incubations contained form-selective substrates [chlorzoxazone (CZX), 100 $\mu$M; $\alpha$-naphthoflavone, 50 $\mu$M; and ketoconazole, 60 $\mu$M] and 0.9 mM NADPH. TCE metabolism was assessed as previously reported (Lipscomb *et al.*, 1998b). Donor demographics are presented in Table 2.

*CYP450 form-specific activities.* CYP450 form-specific activities of CYP1A1/1A2, CYP2E1, and CYP3A were estimated by determining phenacetin *O*-deethylase (PAD; CYP1A), 4-nitrophenol hydroxylase, or chlorzoxazone hydroxylase for CYP2E1 and either erythromycin *N*-demethylase or testosterone 6$\beta$-hydroxylase for CYP3A as previously described (Snawder *et al.*, 1994). All reactions were initiated by the addition of an NADPH-regenerating system (in 0.1 M potassium phosphate buffer) containing 14 mmol glucose 6-phosphate, 0.66 mmol NADP+, and 3 units glucose-6-phosphate dehydrogenase.

*Determination of individual CYP450 forms in human liver samples.* Content of specific CYP450 forms were estimated by a direct ELISA. Briefly, 0.5 $\mu$g of microsomal protein/well was plated to microtiter plates (carbonate–bicarbonate buffer, pH 9.0) along with microsomes containing a known quantity of the CYP450 form (1000 fmol Gentest–1 fmol CYP450/50 $\mu$l) of interest for a standard curve. Plates were incubated overnight at 4° C and the plating solution was removed the following morning. One hundred microliters of 50% fetal bovine serum (FBS) in PBS was added as a blocking agent and plates were incubated for 1 h at 37°C. The blocking agent was removed and plates were washed three times (TBS–Tween, 10% FBS) and incubated at 37°C for 1 h with primary antibody (anti-CYP1A, CYP2B, CYP2C, CYP2E1, CYP3A; Gentest, Woburn, MA). Primary antibody was removed and plates were washed and were then incubated 1 h with 200 $\mu$l/well of anti-goat–alkaline phosphate conjugate. The secondary antibody was removed and plates were washed and 150 $\mu$l of K-Gold premixed ELISA phosphatase substrate (ELISA Technologies, Lexington, KY) was added to each well. After 30 min the plate was read at 405 nm. Absorbance of sample-containing wells was compared to a standard curve. Values were expressed as pmol CYP450 form per milligram of protein.

*Statistical analysis.* Data were evaluated by Student's *t* test and analysis of variance with post hoc evaluation of differences by Student–Newman–Keuls test ($P < 0.05$). The tests were performed using a SAS General Linear Models program (SAS Institute, Cary,

**TABLE 2**
**Donor Demographics**

| Age, race, sex[a] | C.O.D.[b] | Alcohol consumption | Cigarette smoking |
|---|---|---|---|
| 41 C F | GSW | Social | 1 ppd |
| 42 C F | SAH | 2–3 drinks/day | |
| 51 C F | SAH | Social | 1 ppd × 35 years |
| 50 C F | CHI | NR | NR |
| 43 B F | SAH | NR | NR |
| 26 C F | CHI | Moderate | 1 ppd × 11 years |
| 22 H F | SAH | NR | NR |
| 50 C F | SAH | Social | NR |
| 60 C F | SAH | NR | NR |
| 36 C F | SAH | Social | 1–2 ppd × 16 years |
| 47 C F | SAH | NR | 2 ppd |
| 50 C F | SAH | NR | Smoker |
| 45 C F | SAH | Yes | 1 ppd × 30 years |
| 50 C F | SAH | NR | NR |
| 40 C F | CHI | Yes | 2 ppd × 20 years |
| 65 C F | SAH | Social | 1 ppd × 30 years |
| 46 C F | SAH | NR | NR |
| 32 C M | GSW | Social | 1 ppd |
| 32 C M | CHI | NR | 1 ppd × 10 years |
| 42 C M | CHI | Social | 1 ppd × 25 years |
| 51 C M | CHI | 2–3 drinks/day | NR |
| 48 C M | Anoxia | NR | NR |
| 24 C M | Anoxia | NR | NR |
| 39 C M | Anoxia | NR | NR |
| 47 C M | GSW | Social | 1 ppd |
| 25 C M | GSW | Yes | NR |
| 60 C M | Fall | Occasional | 2.5 ppd × 25 years |
| 52 C M | SAH | Heavy | 2–3 ppd |
| 42 C M | SAH | Heavy | 1 ppd |
| 40 C M | CHI | Yes | NR |
| 43 H M | SAH | NR | NR |
| 24 H M | MVA | Social | NR |
| 22 C M | CHI | Yes | Smoker |
| 47 C M | Anoxia | Heavy | NR |
| 31 C M | Stroke | Yes | 1 ppd |
| 49 C M | SAH | Yes | NR |
| 47 B M | SAH | Yes | 1 ppd × 20 years |
| 23 C M | CHI | Social | 1 ppd × 30 years |
| 38 C M | SAH | NR | NR |

[a] Samples are identified by age in years, ethnic background, sex, where B, black; C, caucasian; H, hispanic; M, male; F, female. Cigarette smoking is reported in packs per day (ppd) when quantified. Ethanol consumption was subjectively reported. NR, a negative response was given for cigarettes and/or ethanol.

[b] C.O.D., cause of death, where GSW, gunshot wound; SAH, subarachnoid hemorrhage; CHI, closed head injury; and MVA, motor vehicle accident.

NC), by Sigmastat (Jandel Scientific, San Rafael, CA) or by Microsoft Excel on an IBM personal computer.

*Distribution fitting methods.* Summary statistics and histograms were generated for each of the variables. To estimate the fit between apparent and actual distributions, these measures provided information on the basic shape and input parameters to use for the proposed distribution. QQ plots were used to assess whether data had a particular distribution. This method plots the ordered data on the *x*-axis against the expected values of the trial distribution on the *y*-axis. If the trial distribution fairly represents the data, then the plot will approximate a straight line. Several plausible distributions were tried; selection of the appropriate distribution for each data set was made visually.

## RESULTS

Total CYP450, assessed by spectral analysis, in this sample set range from 0.13 to 0.82 nmol/mg microsomal protein (Fig. 1). Mean content of total P450 was 0.447 ± 0.171 nmol/mg microsomal protein. Mean concentrations of total P450 and individual forms are summarized in Table 3 and the statistical measures of distribution are displayed in Table 4. Of the forms evaluated, CYP3A protein appears predominant (pmol P450/mg microsomal protein) in human hepatic microsomes. With respect to microsomal protein, CYP2C forms and CYP2E1 proteins are expressed on average at approximately 45 and 37% the levels of CYP3A (Table 3). Expression of CYPs 1A, 2B, 2C6, 2C11, 2E1, and 3A ranged 36-, 13-, 11-, 2-, 12-, and 22-fold, respectively, between the highest and lowest values measured in these human microsomes (Table 4). No sex-dependent differences were observed in levels of protein expression or activity of any forms among the samples tested (data not shown). Further evaluation of the fractional composition of total P450 by the forms studied revealed no significant differences between sexes (Table 5). Only CYP3A protein content was correlated to total CYP450 content (data not shown).

To confirm the validity of the ELISA method for

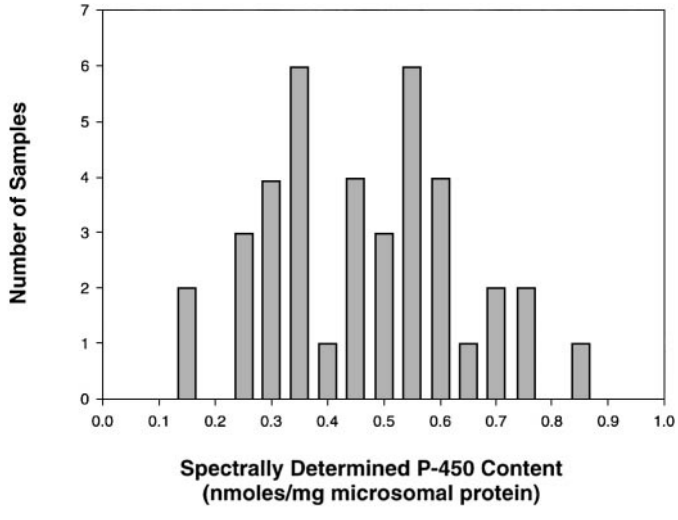


**FIG. 1.** Total CYP450 in liver microsomal samples from 40 human donors. Values in this sample ranged from 0.13 to 0.82 with a mean and SD of 0.45 ± 0.17 nmol P450/mg microsomal protein.

**TABLE 3**
**Concentration of Individual CYP450 Forms in Human Liver Microsomes Determined by ELISA ($n$ = 40)**

| CYP450 enzyme | Specific content (pmol/mg protein) | Pecentage of total spectral CYP450 |
|---|---|---|
| Total CYP450 (spectral determination) | 446.50 ± 171.40 | 100.00 |
| Total CYP450 (sum of forms by ELISA) | 303.07 ± 173.59 | 69.27 ± 20.86[a] |
| CYP1A[b] | 42.43 ± 26.54 | 7.98 ± 5.51 |
| CYP2B6[c] | 2.71 ± 1.85 | 0.58 ± 0.38 |
| CYP2C[d] | 63.61 ± 15.99 | 14.39 ± 5.66 |
| CYP2E1[e] | 52.20 ± 24.66 | 12.91 ± 6.27 |
| CYP3A[f] | 141.82 ± 104.55 | 30.64 ± 16.29 |

[a] $n$ = 23; 49.52 ± 16.96% was accounted for in the remaining 17 samples in which only CYP1A, 2E1, and 3A forms were quantified.
[b] CYP1A, sum of CYP1A1 and CYP1A2; $n$ = 40.
[c] CYP2B6, $n$ = 23.
[d] CYP2C, sum of CYP2C8, CYP2C9, CYP2C18, and CYP2C19; $n$ = 23.
[e] CYP2E1, $n$ = 40.
[f] CYP3A, sum of CYP3A4 and CYP3A5; $n$ = 40.

**TABLE 5**
**Fractional Composition of Cytochrome P450 in Human Hepatic Microsomes**

| CYP Form | Males | Females |
|---|---|---|
| 1A | 7.89 ± 4.42 (22) | 8.08 ± 6.75 (18) |
| 2B | 0.52 ± 0.32 (14) | 0.67 ± 0.47 (8) |
| 2C6[a] | 12.34 ± 5.78 (14) | 12.24 ± 7.79 (9) |
| 2C11[b] | 15.89 ± 5.48 (14) | 17.45 ± 5.87 (9) |
| $2C_{TOTAL}$[c] | 15.15 ± 5.34 (14) | 14.77 ± 6.42 (9) |
| 2E1 | 13.40 ± 6.93 (22) | 12.32 ± 5.26 (18) |
| 3A | 31.73 ± 17.56 (22) | 29.31 ± 14.98 (18) |

*Note.* Data are presented as the percentage of spectrally determined CYP accounted for by individual forms CYP, mean ± SD ($n$).
[a,b] Data were obtained from separate incubations of antibodies against CYP2C6 and CYP2C11 with microsomal protein.
[c] $2C_{TOTAL}$ was determined by mixing the two 2C-directed antibodies above in equal quantities and incubating the antibody mixture with microsomal protein.

quantitative determination of specific CYP450 forms in human hepatic microsomes, levels of specific CYP450 proteins were compared to microsomal metabolic activities that have been recognized as specific for those forms. As seen in Fig. 2, CYP1A protein levels positively correlated to PAD activity, CYP2E1 protein levels positively correlated to CZX-OH activity, and levels of CYP3A protein positively correlate to testosterone 6$\beta$-hydroxylase activity.

Total CYP450 appeared to fit a normal distribution; a mean of 0.45, standard deviation of 0.17 nmol P450/mg microsomal protein, and a variance of 0.03 were observed (Fig. 3A). The QQ plot comparing a normal distribution to the data sets for CYP1A, CYP2B, CYP2C, CYP2E1, and CYP3A forms appeared to simulate a straight line, indicating relative agreement with a normal distribution (Fig. 3). For the variable CYP1A1/2 the QQ plot for a lognormal distribution demonstrated an S-shaped curve. The variable was fit well using a $\beta$ distribution with the shape parameters $\alpha$ and $\beta$ estimated as 1.1 and 1.8, respectively. For the variable CYP2E1, a lognormal distribution was used with relative success. This variable was estimated to be normally distributed with a mean of 1.67 and a variance of 0.04. For the variable CYP3A, the QQ plot for a lognormal distribution demonstrated an S-shaped curve with outliers apparent in the upper tail. The variable was fit well using a $\beta$ distribution with the shape parameters $\alpha$ and $\beta$ estimated as 0.9 and 1.94, respectively.

To examine whether this sample size was limiting in

**TABLE 4**
**Summary Statistics for Total CYP450, CYP1A1/2, CYP2B, CYP2C, CYP2E1, and CYP3A**

| Total | CYP450[a] | CYP1A1/2 | CYP2B | CYP2C | CYP2R1 | CYP3A |
|---|---|---|---|---|---|---|
| Minimum | 0.13 | 3.00 | 0.5 | 32 | 11.00 | 19.70 |
| First quartile | 0.31 | 22.48 | 1.09 | 52.5 | 35.50 | 55.99 |
| Mean | 0.45 | 41.29 | 2.71 | 63.61 | 52.38 | 141.80 |
| Median | 0.46 | 34.00 | 2.24 | 61.5 | 49.00 | 118.50 |
| Third quartile | 0.56 | 52.90 | 4.32 | 74.00 | 65.25 | 234.00 |
| Maximum | 0.82 | 109.00 | 6.48 | 95 | 130.00 | 440.00 |
| Variance | 0.03 | 718.14 | 3.41 | 255.75 | 588.11 | 10931.95 |
| SD | 0.17 | 26.80 | 1.84 | 15.99 | 24.25 | 104.65 |

[a] Data describing total P450 are presented as nmol P450/mg microsomal protein; data describing individual CYP forms are presented as pmol CYP form/mg microsomal protein.
*Note.* QQ plots (see Fig. 3) were used to verify distribution and determine goodness of fit to a normal distribution curve. This method plots the ordered data on the $x$-axis against the expected values of the trial distribution on the $y$-axis. When the trial distribution fairly represents the data, the plot will represent a straight line. Several plausible distributions were tried; selection of the appropriate distribution for each data set was made by visual inspection.

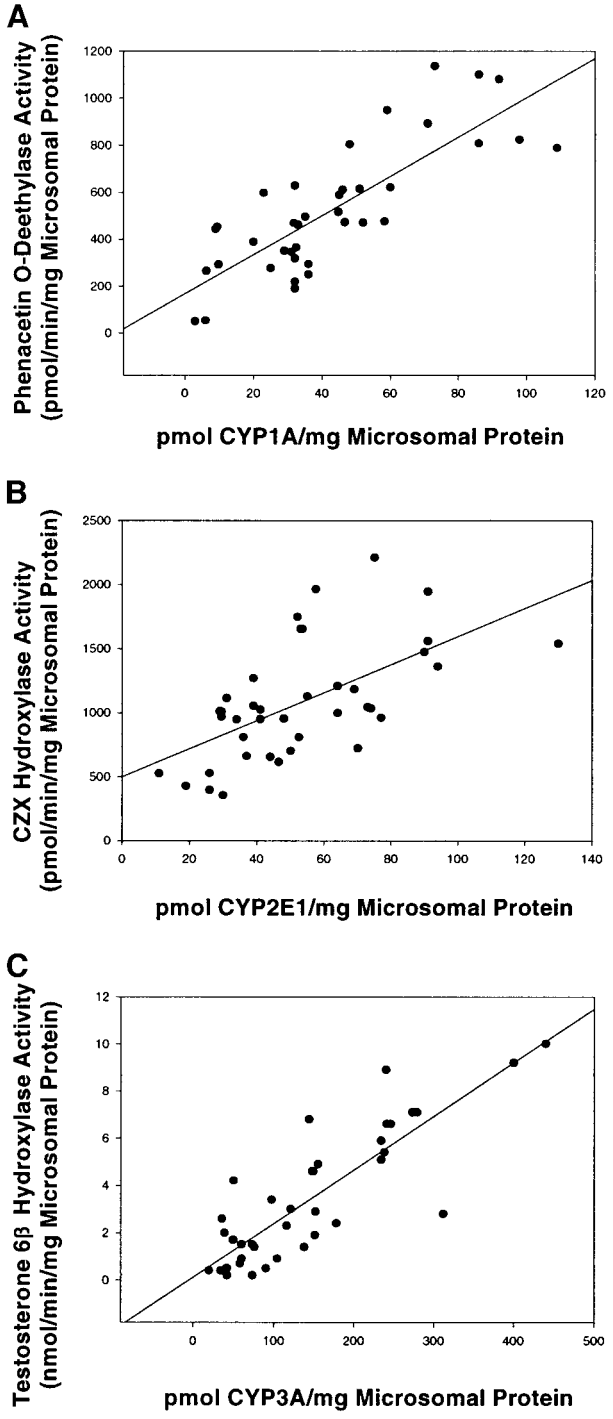


**FIG. 2.** Correlation of ELISA-determined CYP450 expression with characteristic activities. (A) CYP1A protein levels positively correlated ($r^2$ = 0.650) with PAD activity; (B) CYP2E1 protein levels positively correlated ($r^2$ = 0.360) with CZX-OH activity; and (C) levels of CYP3A positively correlated ($r^2$ = 0.724) with testosterone 6$\beta$-hydroxylase activity$\beta$.

discerning biological variations of CYP forms known to be altered by specific environmental or lifestyle factors, we assessed the expression and activity of a form for which donor-specific data were available on modifying factors. The samples were divided into those for which ethanol consumption was reported as negative and those for which ethanol consumption was reported. Results presented in Table 6 indicate a significant ($P < 0.05$) 25% elevation of CYP2E1-dependent chlorzoxazone hydroxylation in liver samples obtained from drinkers. However, while the ELISA-detected expression of CYP2E1 protein in these liver microsome samples obtained from donors who reported drinking ethanol was elevated 26% above the levels in samples derived from nondrinkers, the difference here did not attain the level of statistical significance. The specific activity of CYP2E1 toward CZX was also not appreciably different between nondrinkers (23.2 ± 10.4 pmol/min/pmol CYP2E1) and drinkers (21.8 ± 7.9 pmol/min/pmol CYP2E1).

Specific CYP450 levels in human samples were correlated to metabolic activities toward TCE. Previous work in our laboratory demonstrated that a pooled sample of human hepatic microsomes possessed a $K_m$ for the production of chloral hydrate (CH) or trichloroethanol (TCOH) of 125 $\mu$M. The capacity to metabolize TCE was not correlated to total CYP450 content in the samples tested. Samples containing high amounts of CYP2E1 displayed the strongest correlation toward CH as a product of TCE metabolism. Among other forms examined, only CYP2B displayed a consistent correlation between protein content and CH formation (Table 7). When TCOH production from TCE was measured in these samples at a substrate concentration of 125 $\mu$M, TCOH production most strongly correlated to CYP2B content; CYP450 forms CYP2C and CYP2E1 were also positively correlated (Table 8).

## DISCUSSION

The development of large, well-characterized banks of human tissue samples is advantageous in that critical metabolic and biochemical factors which mediate human interindividual variance in the response to toxic chemicals may be quantified. Because of the metabolic mediation of toxicity of a large number of occupationally and environmentally important chemicals, we sought to develop a procedure which could rapidly and reliably quantify these enzyme forms in large numbers of samples. In contrast to Western blotting techniques, ELISA methods provide the following advantages; increased sensitivity (pg vs $\mu$g), smaller sample size (0.5 vs 5–50 $\mu$g protein), greater speed to completion (4 h vs 2 days), greater efficiency (test up to 36 samples at once), lower equipment needs, and fewer technical challenges. ELISA methods have been used previously to quantify specific CYP450 forms in liver microsomes from dogs (Bandiera *et al.*, 1986; Eguchi *et al.*, 1996; Nishibe *et al.*, 1998), rats (Roe *et al.*, 1998),

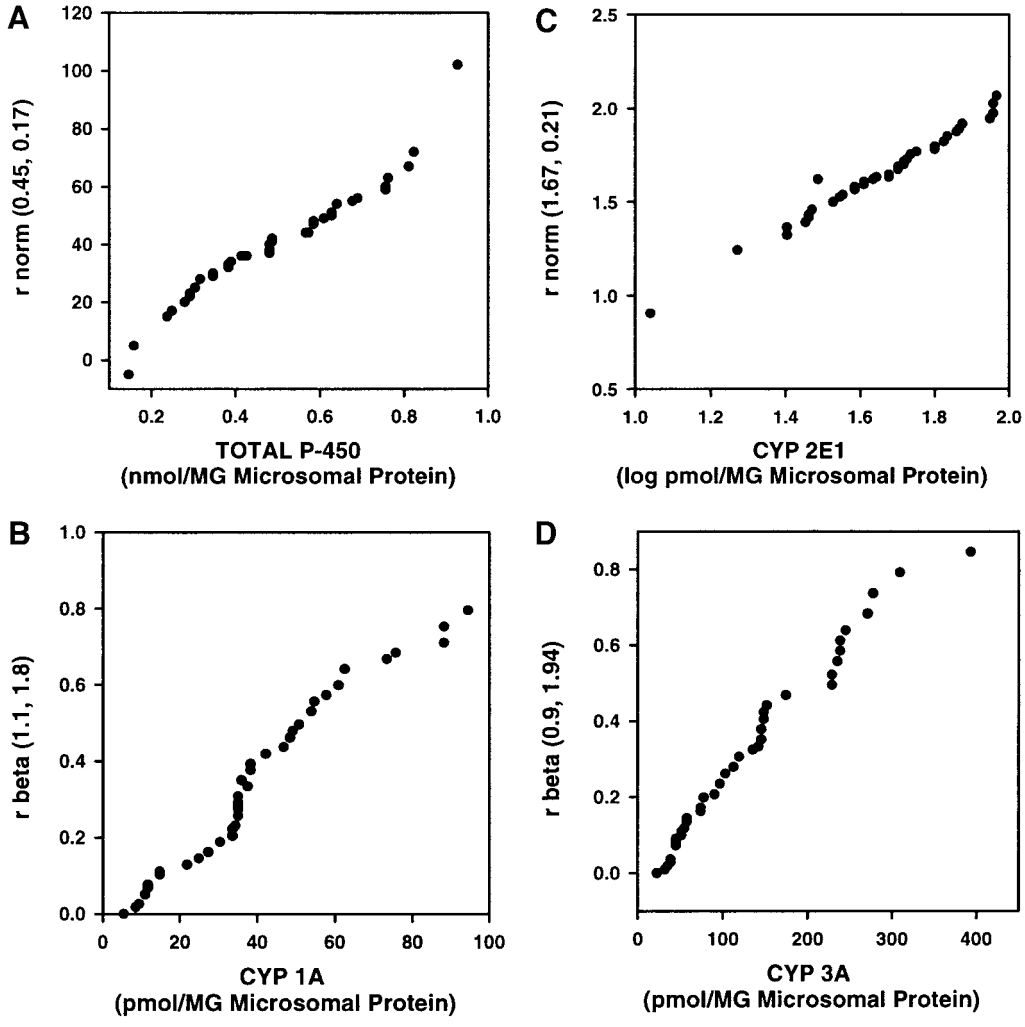


**FIG. 3.** QQ plots demonstrating goodness of fit of distributions to sample data sets. Numbers in parentheses along the *y*-axis represent the mean and variance for normal distributions and $\alpha$ and $\beta$ for $\beta$ distributions. Each graph presents the correlation between the actual data and the predictions of distributed values determined from 1000 simulations of the tested distribution. Actual data are represented by position along the *x*-axis, and the points on the graphs depict the intersection with the predicted values plotted on the *y*-axis. Predicted values are those from normal or $\beta$ distributions which were based on parameters describing the mean and variance for normal distributions (total and logs of CYP2E1, A and C, respectively) or $\alpha$ and $\beta$ for beta distributions (CYP1A and CYP3A forms, B and D, respectively). Mean, variance, $\alpha$, and $\beta$ were estimated from the actual data sets for inclusion in the simulations. Linear correlations, as those demonstrated here, indicate close agreement between observed values and those predicted by the postulated distribution type. (A) Normal distribution of total P450 nmol P450/mg microsomal protein; (B) normal ($\beta$) distribution of CYP1A, pmol CYP1A/mg microsomal protein; (C) normal distribution of log of pmol CYP2E1/mg microsomal protein; and (D) normal ($\beta$) distribution of CYP3A forms, pmolCYP3A/mg microsomal protein.

and mice (Muto *et al.*, 1997; Roe *et al.*, 1999). With the availability of antibodies of great sensitivity and heterologous CYPP450 expression systems, this technique should prove to be a valuable tool in quantifying the content of specific CYP450 forms in human tissue banks as performed in the present study. The use of these well-characterized tissue banks in specific toxicologic and risk assessment studies may be readily accomplished.

The acceptance of PBPK modeling efforts as applied in human health risk assessments has provided one basis for the improvement of human health risk estimation. This approach and others likely to generate specific information on mechanisms of action and modifying factors of toxicity have been identified in the EPA's 1996 "Proposed Guidelines for the Assessment of Carcinogens" as factors which can be used as modifying factors in the estimation of human health risk. Dividing $UF_H$ into the components of pharmacokinetic uncertainty and pharmacodynamic uncertainty may render their elucidation more amenable to testing and clarify sources of uncertainty more precisely. For compounds which exert their toxicity in the liver through a bioactivated metabolite, both the pharmacokinetic and the pharmacodynamic

**TABLE 6**
**Impact of Ethanol Consumption on Cytochrome P450 Expression and Activity**

| | Total P450 | | CYP2E1 protein | | CZX activity | |
|---|---|---|---|---|---|---|
| | Nondrinkers | Drinkers | Nondrinkers | Drinkers | Nondrinkers | Drinkers |
| Mean | 0.436 | 0.454 | 46.1 | 58.1 | 940 | 1178 |
| SD | 0.199 | 0.152 | 18.9 | 27.2 | 352 | 494 |
| Variance | 0.039 | 0.023 | 58 | 739 | 1178 | 244681 |
| *P* value | 0.382 | | 0.053 | | 0.044 | |

*Note.* Data are presented as nmol spectrally determined P450/mg microsomal protein, as pmol CYP2E1/mg microsomal protein, and as pmol CZX hydroxylated/min/mg microsomal protein. $n = 17$ nondrinkers; $n = 23$ drinkers.

processes may be mediated through differences in metabolism. Several low-molecular-weight volatile organic chemicals are bioactivated by the same enzyme system, CYP2E1, and produce liver toxicity. Thus, refined and well-documented estimates of the human interindividual differences in this, and related, enzyme systems can be used in the estimation of human interindividual differences in the distribution, metabolism, and elimination of several chemicals.

In an earlier study (Lipscomb *et al.*, 1997), we evaluated the interindividual variance in the hepatic microsome-mediated conversion of a common groundwater contaminant, TCE, to metabolites thought to mediate some of TCE's toxicity. In that report, we demonstrated, though not surprisingly, a high degree of correlation with activity toward a substrate (CZX) preferentially metabolized by CYP2E1 in a sample of 23 microsome samples obtained from adult human organ donors. Through studies with isolated human hepatocytes (Lipscomb *et al.*, 1998a), we demonstrated that the range of apparent $V_{max}$ values for TCE mimicked the range for TCE apparent $V_{max}$ values obtained from human hepatic microsomes (sevenfold). While neither of these results alone can be used to estimate the human interindividual variance in the pharmacokinetics of TCE at the level of the intact human, additional studies with a limited number of paired human samples ($n = 4$) produced data useful in estimating the distribution of microsomal protein to the intact liver and to isolated hepatocytes which were used to successfully estimate whole body apparent $V_{max}$ values for TCE (Lipscomb *et al.*, 1998a). These data agreed remarkably well with estimates produced from validated PBPK studies in humans (Allen and Fisher, 1993; Fisher *et al.*, 1998). A sensitivity analysis performed on the PBPK model identified the apparent $V_{max}$ for TCE and microsomal protein distribution as having the highest impact (compared with blood:air partitioning, volume of the fat compartment, blood flows, etc.) on the prediction of kinetics, demonstrated as the toxicologically important "amount metabolized." Because the metabolism of TCE and other compounds can be linked directly to individual CYP forms, the expression of these forms in microsomes and intact tissues can lead to more refined extrapolations of *in vitro* determined metabolic rates to intact tissues and to the whole organism. This approach demonstrates the applicability of data such as those presented in this report in the estimation of human interindividual variance, when such data represent the variance about a demonstrated and critical step in mediating the human toxicologic response.

Because of the diverse nature of compounds encountered as environmentally important pollutants and in-

**TABLE 7**
**Regression of Individual CYP450 Forms in Human Liver Microsomes (Determined by ELISA) against Chloral Hydrate Formation From Trichloroethylene**

| CYP450 form | Correlation coefficient ($R^2$) | Probability level (*P* value) |
|---|---|---|
| CYP1A[a] | 0.121 (0.015) | 0.4560 |
| CYP2B6 | 0.470 (0.229) | 0.0240 |
| CYP2C[b] | 0.384 (0.147) | 0.0700 |
| CYP2E1 | 0.654 (0.427) | 0.0002 |
| CYP3A[c] | 0.277 (0.077) | 0.0966 |

[a] CYP1A, sum of CYP1A1 and CYP1A2.
[b] CYP2C, sum of CYP2C8, CYP2C9, CYP2C18, and CYP2C19.
[c] CYP3A, sum of CYP3A4 and CYP3A5.

**TABLE 8**
**Regression of Individual CYP450 Forms in Human Liver Microsomes (Determined by ELISA) against Trichloroethanol Formation From Trichloroethylene**

| CYP450 form | Correlation coefficient ($R^2$) | Probability level (*P* value) |
|---|---|---|
| CYP1A[a] | 0.135 (0.018) | 0.4690 |
| CYP2B6 | 0.774 (0.599) | 0.0007 |
| CYP2C[b] | 0.572 (0.328) | 0.0258 |
| CYP2E1 | 0.403 (0.163) | 0.0301 |
| CYP3A[c] | 0.338 (0.114) | 0.0728 |

[a] CYP1A, sum of CYP1A1 and CYP1A2.
[b] CYP2C, sum of CYP2C8, CYP2C9, CYP2C18, and CYP2C19.
[c] CYP3A, sum of CYP3A4 and CYP3A5.

dustrial compounds, we have extended our studies to include the estimation of many specific CYP proteins in human liver samples. We have demonstrated the variability in the expression of forms which metabolize several groups of compounds including pesticides, organic solvents, and PAH compounds. Although we had previously correlated TCE metabolic activity with activity toward the substrate CZX, we have increased the confidence which can be placed in quantitatively identifying modifiers of CYP2E1 as modifiers of TCE metabolism (and toxicity) in the human. This report extends the finding by including more samples and by examining authentic CYP protein, rather than an indirect measure of CYP activity (CZX metabolism). Through the development of the procedure to extrapolate TCE metabolic rates into a PBPK model for the human, we have demonstrated that degrees of human interindividual variance in chemical metabolism as measured *in vitro* may be usefully applied to estimate human interindividual variance in a step directly related to the manifestation of toxicity. The extrapolation of the enzyme contents presented in this report may prove equally, if not more, useful in producing a more valid approach to estimating human interindividual variance and reducing pharmacokinetic uncertainty among humans with respect to the multitude of chemicals whose toxicity is mediated by these enzymes.

The attainment of data to fulfill several key research needs would advance this approach. Key metabolic processes should be qualitatively and quantitatively linked with the development of a specific event which is causative in the agent's toxicity, obtaining a greater number of tissue samples will increase the statistical power of the results to a broader segment of the population, and the assessment of tissues from individuals within potentially sensitive subpopulations (e.g., alcoholics, children) will each provide valuable data toward the advancement of this area of risk assessment. By obtaining these and other data, the components of the $UF_H$ will become defined in more detail as the RfD methodology advances. We envision that *in vitro* assays will become increasingly used to identify interindividual differences in pharmacokinetics and pharmacodynamics, two key components of $UF_H$. These data will continue to be used in PBPK models; $UF_H$ could also be defined in a more comprehensive manner through chemical-specific PBPK models. Ideally, biologically based dose–response models will be employed in risk assessment which can incorporate interindividual differences.

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