Cytochrome P450 Enzyme Mapping in Drug Discovery using Corning® Supersomes™ Enzymes

Application Note 467

CORNING

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Introduction

Oxidative drug metabolism via the cytochrome P450 (CYP) system is a principle means of drug clearance. Multiple CYP forms may contribute to clearance of small molecule drugs (MW <1500) and drug-like compounds,[1] with isoforms CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A as those which are primarily responsible for human drug metabolism. The absolute levels and CYP enzyme activities vary substantially among individual CYP forms and among individuals. This variability has been linked to genetic polymorphisms, disease and exposure to pollutants, drugs, herbal supplements and other dietary materials which can either increase or decrease levels of individual or groups of CYPs.[2,3] In contrast, inter-individual CYP activities in animal-model species are generally more consistent as these models are inbred and diet/environmental factors can be rigorously controlled.

In vitro testing for the role of these CYPs in the metabolism of a drug candidate is standard practice in drug discovery and development. Regulatory guidance documents have been developed for this testing[4]. In addition, the prediction of human pharmacokinetics (PK) typically requires testing of the rates of CYP metabolism with *in vitro* systems to determine the proportion of overall clearance that is accounted for by the various CYP forms and other metabolic enzymes.

Corning Supersomes enzymes are recombinantly expressed drug metabolizing enzyme reagents, consisting of microsomes prepared from insect cells infected with a virus engineered to express a CYP isoform. The CYP activity is supported by the presence of its redox partner NADPH cytochrome P450 oxido-reductase (OR) and, in most cases, cytochrome b5. As an *in vitro* reagent, they may be used just like liver microsomes. Other non-CYP drug metabolizing enzymes are available under the Corning Supersomes enzymes brand name including flavin-containing mono-oxygenases (FMOs), UDP-glucuronosyl transferases (UGTs), carboxyl esterases, monoamine oxidases (MAOs), or N-acetyl transferases (NATs).

Generally, the CYP activity in Corning Supersomes enzymes is much higher than pooled human liver microsomes (HLMs). The successful use of Corning Supersomes enzymes for quantitative metabolism studies *in vitro* requires an understanding of the relative rates of catalytic activity found in Corning Supersomes enzymes relative to HLMs.

Why Conduct Enzyme Mapping in Drug Discovery?

Enzyme mapping in an ADME context typically refers to an assessment of the identity, number, and relative contribution of drug metabolizing enzymes involved in metabolic clearance. Drugs that are cleared principally by one metabolic pathway are at higher risk to have the pathway inhibited by a co-medication and of becoming a so-called "victim" of a PK drug-drug interaction event. In addition, since clearance is proportional to the amount of functional enzyme in an individual, therapeutic efficacy may also be affected. In turn, this can increase risk of drug failures.[5] It follows, therefore, that the more enzymes or pathways that contribute to clearance, the less interpatient variability and susceptibility to drug-drug interactions.

The purpose of this Application Note is to provide overviews of approaches to assessing contribution of individual CYP isoforms to a drug's clearance at the stage of drug discovery. At the drug discovery phase, a definitive test is rarely conducted. This is because definitive phenotyping a given reaction requires a thorough understanding of metabolic pathways and radiolabeled drug or synthetic metabolite standards are usually not available. However, it is often desirable to "screen" for desirable properties (e.g. multiple pathways) in drug discovery and lead optimization. Because radiolabeled material and authentic metabolite standards are not readily available in discovery, the approach of relative quantitation by assessing "substrate depletion" is typical and will be the focus of this Application Note.

In a substrate depletion experiment, the amount of metabolism is assessed after various time points (e.g., 0, 5, 10, 20, 30, 45, and 60 minutes) by measuring how much substrate is remaining relative to that found at the initiation of the incubation. Since only the substrate is quantified, the amount of metabolism is equivalent to the sum of all metabolites formed. Compared to measuring formation of metabolites, this is an imperfect approach. This is because substrate depletion is inherently less sensitive because one must subtract two large numbers, making the difference imprecise. In addition, one cannot determine which and how many metabolites comprise the substrate depleted. Time and resources permitting, one can monitor suspected metabolites; however, quantitation of peak areas does not necessarily equate to metabolite abundance because ionization efficiencies may differ.

Procedures for a Substrate Depletion Experiment

Materials and methods

◗ Potassium phosphate buffer, 0.5 M, pH 7.4 (Corning Cat. No. 451201)

◗ NADPH regenerating system, Solution A (Corning Cat. No. 451220); Solution B (Corning Cat. No. 451200)—thawed at 37°C and kept on ice

◗ Corning® Supersomes™ enzymes (multiple catalog numbers, Table 3)—thawed at 37°C and kept on ice

Phosphate buffer can be combined with Corning Supersomes enzymes and an NADPH regenerating system to make a convenient assay mix for measuring activity of CYP enzymes as outlined in Table 1. Solution A is a 20X concentrate of NADP+, Glucose 6-phosphate (G6P) and $MgCl_2$. Solution B is a 100X concentrate of Glucose 6-phosphate dehydrogenase (G6PDH). The table below is an example showing how the three products can be combined to make a standard CYP reaction assay mix. Prepare the NADPH regenerating system first, by combining solutions A and B, keeping solutions chilled or on ice. Mix with buffer and warm to 37°C. Add the test article solution. A typical substrate concentration is 1 µM. Initiate the reaction by the addition of Corning Supersomes enzymes. The volume of Corning Supersomes enzymes to be added may vary depending on strategy, as discussed below.

Assay Reagent	Volumes (µl) for a 1400 µl incubation volume	Final Concentrations	Volumes for ten 1400 µl reactions
0.5 M KPO₄ (Corning Cat. No. 451201)	280	100 mM	2800
Solution A (Cat. No. 451220) (20X)	70	1.3 mM NADP⁺, 3.3. mM G6P, 3.3 mM MgCl	700
Solution B (Cat. No. 451200) (100X)	14	0.4 Units/mL G6PDH	140
H₂O	895	—	8950
1 mM substrate (dissolved in DMSO)	1.4	1 µM	14
Corning Supersomes enzymes (1 pmol/µl)	140	100 pmol/mL	1400

Table 1. Standard assay reaction components and volume for Corning Supersomes enzymes.

Time (X)	Percent (Y)	LN%
0	100	4.61
5	94	4.54
10	90	4.50
20	78	4.36
30	60	4.09
45	55	4.01
60	50	3.91

Table 2. Example results of a substrate depletion experiment with Corning Supersomes enzymes

Incubations may be conducted in duplicate with an initial volume of 1.4 mL. At multiple incubation times (0, 5, 10, 20, 30, 45, and 60 minutes), 0.1 mL is withdrawn and mixed with 0.1 mL acetonitrile to stop the reaction and precipitate the protein. The acetonitrile stop solution may contain an internal standard, such as 0.1 µM labetalol, to normalize the response of the mass spectrometer. Labetalol is advantageous as an internal standard as it can be easily detected when the mass spectrometer is operating in either positive or negative ion detection mode. The samples are centrifuged (e.g. 4000 g, 15 minutes, 20°C or less) and stored at -20°C until analysis.

Data analysis – determination of CL$_{int}$. To determine the value of *in vitro* intrinsic clearance (CL$_{int}$), peak areas of parent drug at each time point are tabulated and expressed as a percent of the peak area at the zero minute time point, which represents 100% (Table 2). Data may be plotted as in Figure 1.

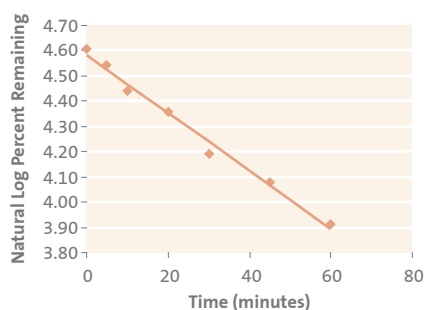**Figure 1.** Plot of incubation time vs. natural log percent remaining

In vitro *half-life (t1/2) may be determined using the equation: t1/2 = ln2/-k, where k represents the terminal elimination rate constant and is calculated as the negative slope of the line defined by the linear regression of the natural log loss of substrate and incubation time. In the example outlined in Table 2 and Figure 1, slope = -k = -0.0115. The slope may be calculated using the "SLOPE" function in Microsoft® Excel software.*

CL_{int} of a recombinant CYP may then be calculated using the equation below:

$$CL_{int, rCYP} = \frac{0.693}{in\ vitro\ t_{1/2}} \times \frac{mL\ incubation}{pmol\ P450}$$

Where:

mL incubation = the volume of the incubation at T = 0

pmol P450 = the pmol of CYP isoform in the incubation

By definition, when S << K_M, $CL_{int, rCYP}$ = V_{MAX}/K_M and has units of μL/min*pmol (or mL/min*pmol).

In these experiments, the recovery of the test article is typically ignored although it should be recognized that nominal concentrations may not necessarily equate to the unbound concentration.

Choice of enzyme concentration. The choice of the amount of enzyme added to each incubation may vary depending on the strategy employed. For example, an identical amount (such as 100 pmol/mL) of each P450 isoform tested may be used. Alternatively, the amount may be scaled to match relative activity for a specific isoform found in pooled HLM. Using identical amount simplifies the experimental design and can have advantages in standardizing reagent volumes. Conversely, scaling the amounts added helps directly relate the enzyme activity observed in the experiment to that found in HLM. Each approach is considered below.

Standardization by addition of equivalent amounts of each P450 isoform. In this approach, an identical amount of P450 isoform is added, such as 100 pmol/mL. Higher concentrations may be needed for compounds that are highly metabolically stable. For the major P450 isoforms, Corning® Supersomes™ enzymes are formulated at 1 μM thereby permitting a standard volume of reagent to be added. However, Corning Supersomes enzymes differ in protein concentration and it may be desirable to normalize total protein across all CYP isoforms. This can be done by adding control Corning Supersomes enzymes (insect cell protein from mock-transfected cells), normalizing to the isoform contributing the most total protein (or slightly higher). Typically, total protein concentrations are standardized at 0.5 to 2.0 mg/mL. Data analysis may be conducted using relative activity factors (RAF) or intersystem extrapolation factors (ISEF) as described below. In either case, one normalizes the response exhibited by Corning Supersomes enzymes to that of the native enzyme. This is because Corning Supersomes enzymes may yield different rates of activity (usually higher) relative to the individual liver microsomal CYP isoform. Differing activity rates are attributable to differing concentrations of the CYP isoform and its redox partners, which have been optimized to maximize activity.

Data Analysis using the RAF Method

This approach is based on methods developed at Corning Life Sciences.[6] As above, this method employs the use of Corning Supersomes enzymes to determine the relative contributions of a P450 isoform to a given P450 reaction in HLM. In particular, the method helps define the amount of cDNA-expressed holo-enzyme required to obtain the activity of the same enzyme within liver microsomes, thereby relating the activity towards a new chemical entity (NCE) with HLM directly to that in the cDNA-expressed enzyme.

The relative activity factor is defined as:

$$RAF = \frac{V_{MAX, HLM}}{V_{MAX, rCYP}}$$

Where, $V_{MAX, HLM}$ is the velocity of the reaction of interest in HLM expressed in units pmol/min/mg protein and $V_{MAX, rCYP}$ is the velocity of the reaction of interest in Corning Supersomes enzymes data and is expressed in units pmol/min/pmol rCYP. The units of RAF are thus pmol CYP/mg protein. Typically, the activity levels from the data sheet are used, and those activities are obtained generally at saturating substrate concentrations.

Example:

A Corning Supersomes enzyme panel (e.g. n = 10 or more hepatic CYPs) shows that two P450 enzymes metabolize drug Y (CYP3A4 and CYP2C9)

rCYP3A4 activity for Drug Y = 100 pmol/min/pmol

rCYP2C9 activity for Drug Y = 20 pmol/min/pmol

rCYP3A4 activity for probe (testosterone 6ß-hydroxylase) = 150 pmol/min/pmol

rCYP2C9 activity for probe (diclofenac 4'-hydroxylase) = 40 pmol/min/pmol

CYP3A4 catalyzed testosterone 6ß-hydroxylase in HLM = 5300 pmol/min/mg

CYP2C9 catalyzed diclofenac 4'-hydroxylase in HLM = 2900 pmol/min/mg

RAF CYP3A4 = CYP3A4 activity for probe in HLM/rCYP3A4 activity for probe = 5300/150 = 35 pmol rCYP3A4/mg HLM protein

Rate of CYP3A4 Drug Y activity in HLM = RAF CYP3A4 x rCYP3A4 Drug Y activity = 35 x 100 = 3500 pmol/min/mg

RAF CYP2C9 = CYP2C9 activity for probe in HLM/rCYP2C9 activity for probe = 2900/40 = 73 pmol rCYP2C9/mg HLM protein

Rate of CYP2C9 Drug Y activity in HLM = RAF CYP2C9 x rCYP2C9 Drug Y activity = 73 x 20 = 1460 pmol/min/mg

The predicted velocity in HLM, based solely on experiments with Corning® Supersomes™ enzymes, may be expressed as follows:

$$Velocity_{HLM} = \sum_{i=1}^{n} Velocity_{CYPi} \times RAF_{CYPi}$$

For the example with Drug Y above, CYP3A4 contributes 3500 pmol/min/mg and CYP2C9 contributes 1460 pmol/min/mg for a total of 4960 pmol/min/mg.

The percent contribution of CYP3A4 and CYP2C9 to Drug Y activity in HLM is simply:

CYP3A4: 3500/4960 x 100 = 71%
CYP2C9: 1460/4960 x 100 = 29%

Since the V_{MAX} may be unavailable for the drug at the drug discovery stage, the use of intrinsic clearance values can be substituted, where:

Intrinsic Clearance (rCYP) = $CL_{int, rCYP}$ = V_{MAX}/ K_M with units of µL/min*pmol (or mL/min*pmol). CL_{int} values may be obtained by conducting substrate depletion experiments at low substrate concentration where S << K_M and calculated using the equation:

$$CL_{int, rCYP} = \frac{0.693}{in\ vitro\ t_{1/2}} \times \frac{mL\ incubation}{pmol\ P450}$$

In vitro half-life ($t_{1/2}$) may be determined using the equation: $t_{1/2}$ = ln2/-k, where k represents the terminal elimination rate constant and is defined as:

-k = slope of linear regression from natural log percentage substrate remaining versus incubation time.

Thus, RAF can also be expressed as ratios of probe substrate CL_{int} for HLM and rCYP, having the same units as V_{MAX} RAF:

$$RAF_{CL_{int}} = \frac{CL_{int, HLM}}{CL_{int, rCYP}}$$

RAF CL_{int} has units of pmol CYP/mg protein. Confirmation of the RAF result with selective enzyme inhibition in pooled HLM (e.g. Corning UltraPool™ HLM 150) with P450 specific inhibitory antibodies or chemical inhibitors is advisable. For example, exclusive of contributions for other HLM-derived enzymes, a theoretical result of a chemical inhibition experiment may be:

CYP3A4: 1 µM Ketoconazole = ~75% Inhibition in HLM
CYP2C9: 2 µM Sulfaphenazole = ~25% Inhibition in HLM

More extensive discussion on the concept and application of RAF is available in the references listed at the end of this document.

Data Analysis using the ISEF Method

In practice, there are multiple approaches to extrapolating activity from recombinant expression systems to the activity present in HLM.[7,8] Recently, the ISEF method has gained acceptance, based on the growing popularity of simCYP software.[9] The ISEF method combines the RAF and CYP abundance methods for estimating drug clearance.

$$ISEF = \frac{V_{MAX, HLM}}{V_{MAX, rCYP} \times HLM\ CYP\ abundance}$$

Where, $V_{MAX, HLM}$ is the velocity of the probe substrate reaction of interest in HLM expressed in units pmol/min/mg protein and $V_{MAX, rCYP}$ is the velocity of the same reaction of interest in Corning Supersomes enzymes expressed in units pmol/min/ pmol rCYP. The term HLM CYP abundance is the pmol/mg protein in the HLM sample. This value is typically determined by immunochemistry.

ISEF is dimensionless. This is because the units cancel out of the equation. In this case, the turnover number of CYP in HLM is divided by the turnover number in rCYP. The ISEF values take into account the relative abundance of CYP in HLM and consequently permits one to incorporate into the model population variability in metabolism (attributable to factors such as ethnicity, age, or disease state). In turn, this permits modeling of extremes of potential drug interactions. The ISEF is used to estimate NCE clearance using rCYP (Corning Supersomes enzymes) as the enzyme source.

As above, since the V_{MAX} may be unavailable at the drug discovery stage, the use of intrinsic clearance values is usually substituted, where:

$$ISEF = \frac{CL_{int, HLM}}{CL_{int, rCYP} \times HLM\ CYP\ abundance}$$

Each CL_{int} term may be determined using a substrate depletion approach. Typically, the substrate is a probe substrate such as testosterone for CYP3A4, phenacetin for CYP1A2, etc.

Example:

To determine $CL_{int, HLM}$, if the incubation volume is 1400 µL, the concentration of HLM protein in the incubation is 1 mg/mL, and the $t_{1/2}$ is 60 min, then:

$$CL_{int, HLM} = \frac{0.693}{in\ vitro\ t_{1/2}} \times \frac{\mu L\ incubation}{mg\ protein}$$

or,

$$CL_{int, HLM} = \frac{0.693}{60} \times \frac{1400}{1.4}$$

$C_{lint, HLM}$ = 11.55 µL/min*mg protein.

To determine CL$_{int, rCYPi}$, if we assume the volume of incubation is 1400 μL, the pmol P450 in the reaction is 70 pmol and the half life is 60 min, then:

$$CL_{int, rCYP} = \frac{0.693}{in\ vitro\ t_{1/2}} \times \frac{\mu L\ incubation}{pmol\ P450}$$

or,

$$CL_{int, rCYP} = \frac{0.693}{60} \times \frac{1400}{70}$$

and C$_{lint, rCYPi}$ = 0.231 μL/min*pmol P450.

If CYP abundance is 100 pmol CYP3A4/mg microsomal protein, then:

$$ISEF = \frac{11.55\ \mu L/min*mg\ protein}{0.231\ \mu L/min*pmol \times 100\ pmol/mg\ protein} = 0.5$$

One can then use the ISEF value as follows:

If the rate of metabolism of Drug Z using CYP3A4 Corning® Supersomes™ enzymes is found to be 10 pmol metabolite/min/pmol P450, then the expected rate of metabolism in HLMs from a donor with relatively low CYP3A4 (e.g. 20 pmol CYP3A4/mg protein) would be:

10 pmol/min/pmol P450 x 0.5 x 20 pmol/mg = 100 pmol/min/mg protein

For a donor with relatively high CYP3A4 (e.g., 130 pmol/mg protein), then the expected rate of metabolism would be 10 pmol/min/pmol P450 x 0.5 x 130 pmol/mg = 650 pmol/min/mg protein.

Scaling Corning Supersomes Enzymes Volumes to Mimic Liver Microsomal Rates

In contrast to adding standard volumes, Corning Supersomes enzymes may be added in amounts that yield enzyme activity equivalent to that found in pooled HLM for a given probe substrate. This approach offers the following advantages – 1) Ease in interpretation of data. The amount of metabolism observed is more likely to represent rates of metabolism properly apportioned to mean CYP isoform content in pooled HLM; 2)

for low abundant enzymes, the consumption of reagent is less, offering a cost advantage. A variation on this approach is adding Corning Supersomes enzymes amounts targeting twice the enzyme activity present as in the pooled HLM incubations. Since velocity is proportionate to enzyme amounts, this approach is biased towards observing loss of parent. This latter approach is outlined in Table 3. In this case, it had been determined there was significant metabolism in a pilot experiment with HLM using 0.2 mg/mL. Since multiple P450 isoforms may contribute to total metabolism in HLM, the contribution attributable to one P450 isoform will of course be at most 100%. It is prudent, therefore, to choose the HLM protein concentrations and incubation times to base the Corning Supersomes enzymes experimental design that ensures adequate loss of the parent compound (e.g. 50% loss or more) to apportion between the contributing isoforms. For metabolically stable compounds, observing adequate loss can be problematic as indicated earlier. Depending on the precision of your assay, loss of parent of 15% might be considered a minimum to reliably distinguish metabolism from experimental error.

The activity calculation for the cDNA-expressed enzymes may be based on the activity supplied on the batch data sheet for each P450 isoform Corning Supersomes enzymes, and the average HLM activity levels for the corresponding probe substrate provided in Table 4.

A	HLM concentration	0.5 mg/mL
B	Average HLM diclofenac 4'-hydroxylase activity	2900 pmol/(mg min)
C	Diclofenac activity of cDNA-expressed CYP2C9	6905 pmol/(mg min)
D	Activity in HLM incubation	A x B or 1450 pmol/(mL min)
E	Desired activity	2 x D or 2900 pmol/(mL min)
F	Amount of cDNA-expressed CYP2C9 protein	E/C or 0.42 mg/mL

Table 3. Activity calculation for the cDNA-expressed enzymes

Protein concentration may be kept constant within an experiment and standardized by the addition of control microsomes. Control microsomes and buffer only can serve as blanks. Typical enzymes examined and example parameters in a reaction phenotyping experiment are contained in Table 4.

Corning® Supersomes™ Enzyme (Cat. No.)	Lot No.	Reference Reaction	Corning Supersomes Enzyme Activity[1]	Protein Concentration (mg/mL)	Corning Supersomes Enzyme Activity[2]	Enzyme Activity in Corning UltraPool™ HLM 150 Pooled HLMs (lot no. 38289)[2]	Amount of Corning Supersomes Enzymes Protein (mg) per mL to Achieve Twice the Activity in 0.5 mg/mL HLM	Amount of Control Corning Supersomes Enzymes Protein to Normalize to 0.5 mg/mL
cDNA-Expressed CYP1A2 (456203)	41273	Phenacetin O-deethylase	43	9.1	4730	650	0.14	0.36
cDNA-Expressed CYP2C8 (456252)	28681	Paclitaxel 6α-hydroxylase	4.9	7.2	680	260	0.38	0.12
cDNA-Expressed CYP2C9*1 (456258)	41274	Diclofenac 4'-hydroxylase	29	4.2	6910	2900	0.42	0.08
cDNA-Expressed CYP2C19 (456259)	38793	(S)-Mephenytoin 4'-hydroxylase	46	5.0	9200	87	0.009	0.49
cDNA-Expressed CYP2D6*1 (456217)	38273	Bufuralol 1'-hydroxylase	38	7.0	5430	75	0.014	0.49
cDNA-Expressed CYP3A4 (456202)	38275	Testosterone 6β-hydroxylase	220	6.7	32840	5300	0.16	0.34
cDNA-Expressed Control (456200)	44194	NA	ND	5.0	ND	NA	NA	NA

1 - pmol product/(min x pmol P450)
2 - pmol/(mg x min)

Table 4. Major cDNA-Expressed P450 isoforms and activities

Tips

◗ For substrate depletion experiments, keep the final test substance concentration in the incubation well below the K_M (e.g. 10X) to permit robust observations of intrinsic clearance (e.g. V_{MAX}/K_M). Because the K_M may not be known at the discovery stage, a standardized concentration of 1 µM is often chosen. The majority of CYP catalyzed reactions exhibit K_M values above 1 µM. However, if 1 µM is not << K_M, then reaction velocities (and clearance) may be underestimated. Varying the substrate concentration in a substrate depletion assay can also be used to determine K_M value separately.

◗ Acetonitrile, up to 2% final concentration, is a preferred solvent for addition of test articles. This is because most P450 maintain good function in this solvent. However, it is often not as practical as DMSO to dissolve discovery compounds. However, DMSO can be inhibitory to P450 activity and we recommend keeping the concentration at 0.2% or less. To facilitate addition of substrate in small volumes of DMSO, dilute the test article in acetonitrile 5- to 10-fold or use a larger incubation volume. Keep the organic solvent concentration constant for all conditions which are to be compared.

◗ Because total protein (not to be confused with total P450!) may vary between Corning Supersomes enzymes catalog numbers, one may want to standardize total protein with insect cell control protein. The concentration of total protein in the reaction may affect the free fraction of drug candidate.

◗ Occasionally, less abundant CYP enzymes are important in clearance or metabolic events in extrahepatic tissues. Suggested additional enzymes include CYP1A1, 2J2, 4Fs, and FMOs. In addition, drug candidates with functional groups such as amines or hydroxyl groups may be cleared by direct glucuronidation catalyzed by UGT. In this case, Corning Supersomes UGT enzymes may be used to assess the number of types of UGTs involved. The substrate depletion approach may also be carried out with other recombinant drug metabolizing enzymes such as carboxyl esterases, MAOs, or NATs.

Conclusions

The standards of rigor are increasing for accurate assessment of reaction phenotyping at earlier stages in the drug development process. The use of Corning® Supersomes™ enzymes represents a robust approach to phenotyping during drug discovery/lead optimization. Coupled with strategies of using selective chemical or immunoinhibitory antibodies, a complete picture of reaction phenotyping is possible. Additionally, an assessment for drug interaction risk, particularly in population extremes (e.g. null phenotypes) emerges. Drug candidates with multiple pathways of clearance will be at less risk for inappropriate pharmacokinetics. For example, drugs eliminated by a single CYP pathway are at risk for being a "victim" of drug interaction when the patient is administered a co-medication known to inhibit the activity of that CYP enzyme. Similarly, if a CYP with a null phenotype is responsible for 100% of clearance, patients lacking that CYP are at risk for drug accumulation and toxicity.

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Abbreviations:

HLM – Human liver microsomes
rCYP – Recombinant cytochrome P450
G6P – Glucose 6-phosphate
NCE – New chemical entity
NA – Not applicable
ND – Not detectable

Corning acquired the Supersomes™ and UltraPool™ brands.
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