Drug Metabolism: Reaction Phenotyping

Andrew G. Taylor, Ph.D.
Manager, Technical Support for Services
XenoTech

In vitro DDI Services

Drug Metabolism
Reaction phenotyping (CYP & UGT)
Metabolic profiling
Customized studies

Enzyme Induction
In vitro studies in cultured hepatocytes (human and animal)
Ex vivo studies in animals
Toxicity & mechanistic studies

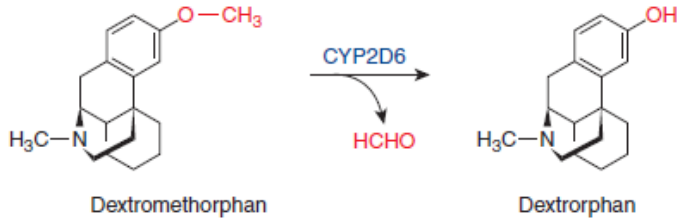
Enzyme Inhibition
Evaluate potential for direct, and metabolism-dependent inhibition(MDI or TDI)
Mechanistic studies (direct or MDI)
Non-CYP enzymes (*e.g.*, UGT, MAO, AO)

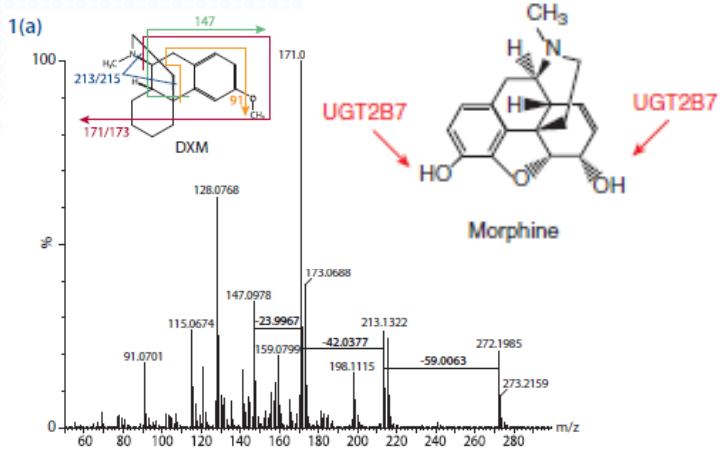
Transporters
FDA and EMA required transporters
In vitro studies in mono-layer cell lines for uptake
Bi-directional assay for efflux transporters
Membrane-based vesicles and ATPase assays

Plasma Protein Binding (PPB)
% Fraction Unbound (f_u)
% Remaining (Plasma Stability)
% Recovery (Mass Balance)

Drug Metabolism Studies

- Reaction phenotyping
  - Identification of enzymes involved in the metabolism of a drug candidate
- Metabolic stability
  - Comparison of metabolic stability and intrinsic clearance among drug candidates (microsomes, S9, hepatocytes)
- Metabolic profiling (AS)
  - Identification of the metabolites
- Species comparison
  - Provides information to select appropriate nonclinical species





Drug Metabolism Studies

It is important to identify the enzymes that significantly contribute to the metabolism of a drug candidate and to evaluate the potential of a drug candidate to be a:

- ***victim*** drug (a drug which is cleared by a main route of elimination)
- ***perpetrator*** drug (a factor or drug which alters the clearance of a victim drug)

Test systems used depend on enzymes to be evaluated (e.g., HLM for CYP, cytosol for Phase II enzymes, S9 or hepatocytes for CYP + Phase II)

CYP Reaction Phenotyping: Requirements

2020 FDA Recommendations:

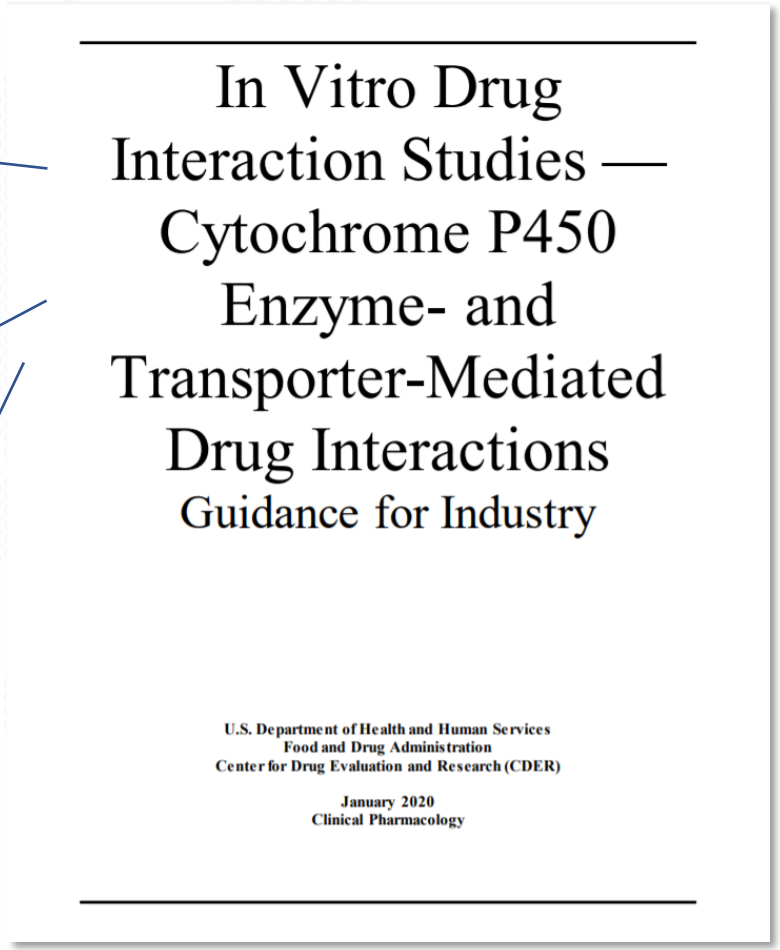
Use validated analytical methods

Triplicate determinations

Perform at least two (rCYP & chemical inhibition) tests

CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A. Other enzymes as needed

Include positive controls



In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

January 2020
Clinical Pharmacology

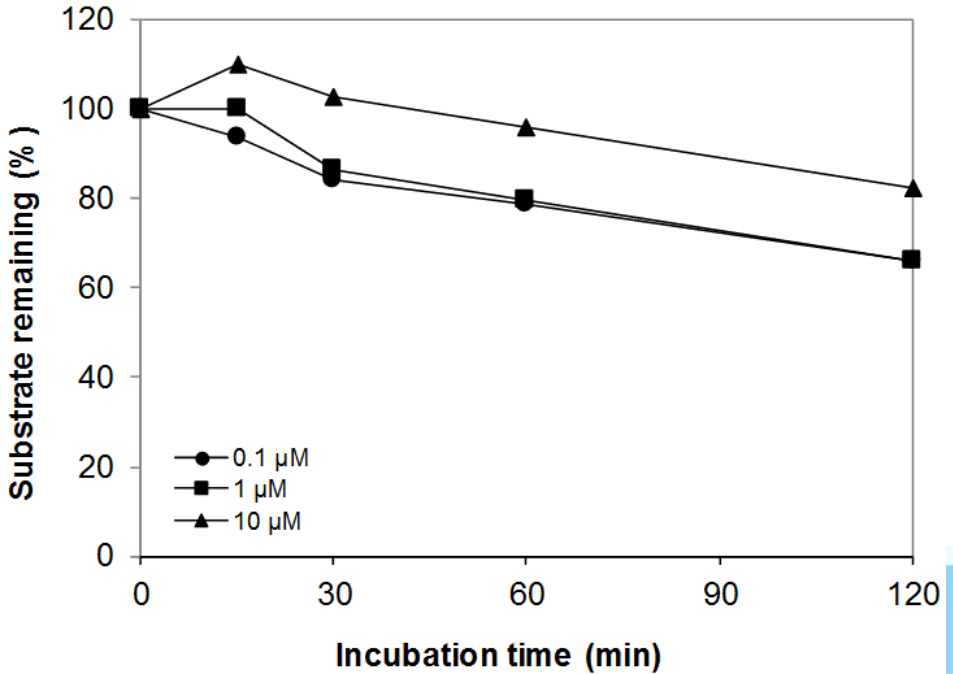
CYP Reaction Phenotyping: Standard Design

- **Preliminary study**
  - Time and protein
- **Optional follow-up, depending on preliminary results and/or sponsor:**
  - rCYP
  - Chemical inhibition (usually HLM)
  - Correlation analysis (rare)

CYP Reaction Phenotyping: Preliminary Evaluation

Design:

- 3 concentrations of test article
- 3 concentrations test system
- 0 + 4 incubation time points



- Establish initial rate conditions with pooled HLM
- Substrate loss (or metabolite formation) is proportional with respect to incubation time and protein concentration
- Incubation times can be long (e.g., 0.5 to 2 hr)

Recombinant CYP (rCYP) & Chemical Inhibition

Two complementary methods

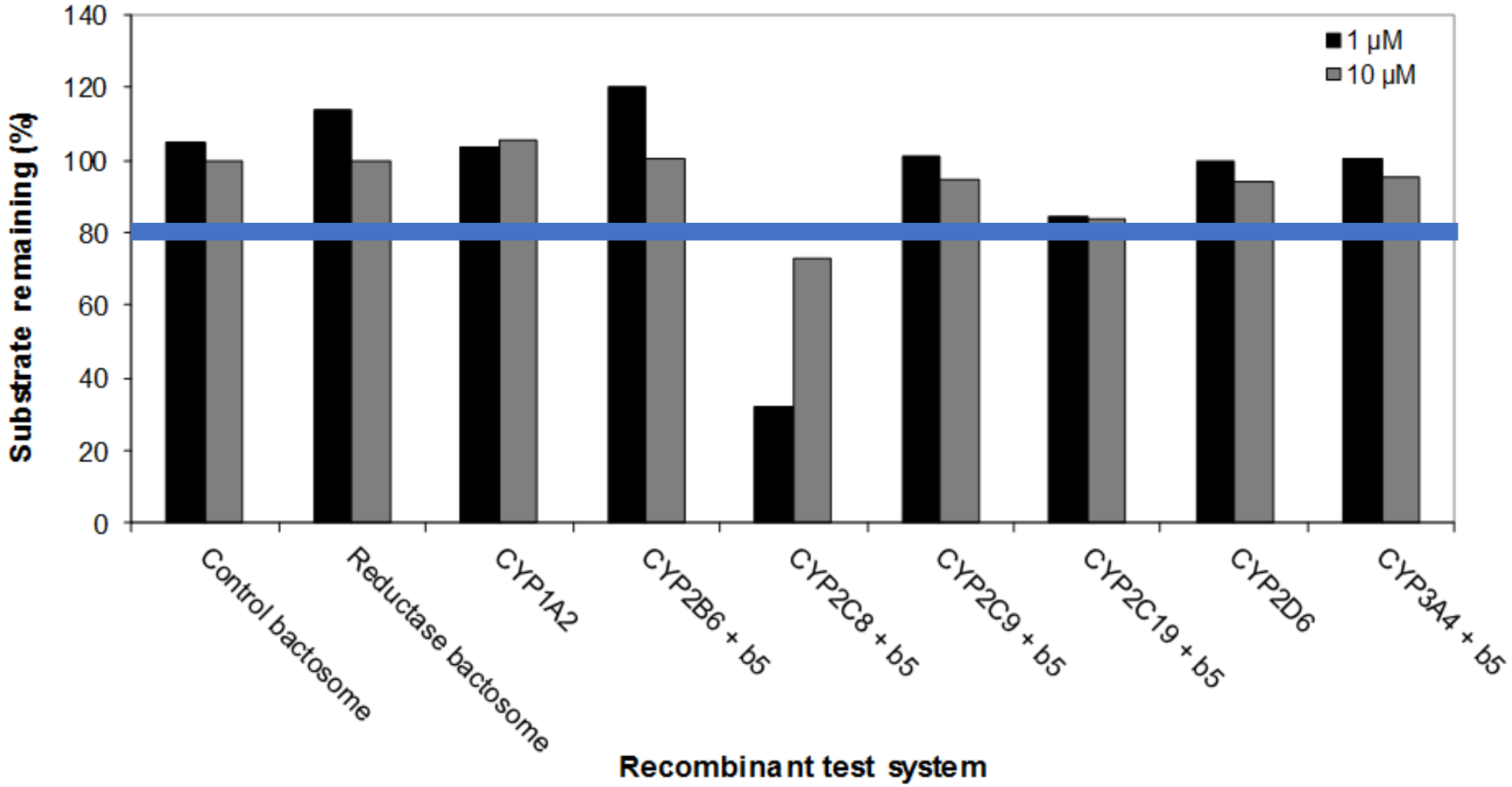
CYP Reaction Phenotyping: Recombinant CYP (rCYP)

- Recombinant enzymes: human enzymes expressed in bacteria or insect cells
- Enzyme expression is different than in vivo (e.g., HLM, hepatocytes)

RECOMBINANT ENZYMES
Cypex

Design:

- 1 or 2 concentrations of test article
- Panel of CYP enzymes (7 standard)
- 1 concentration rCYP (time and protein samples with HLM included for comparison)
- 1 incubation time point

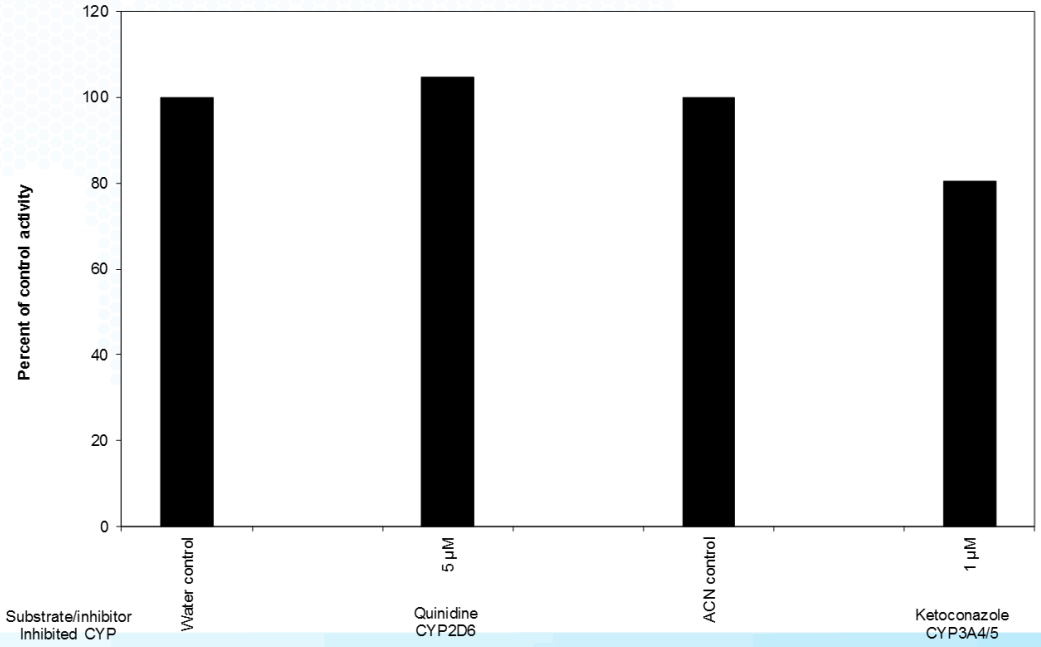

140
120
100
80
60
40
20
0
Substrate remaining (%)
1 µM
10 µM
Control bactosome
Reductase bactosome
CYP1A2
CYP2B6 + b5
CYP2C8 + b5
CYP2C9 + b5
CYP2C19 + b5
CYP2D6
CYP3A4 + b5
Recombinant test system

CYP Reaction Phenotyping: Chemical Inhibition

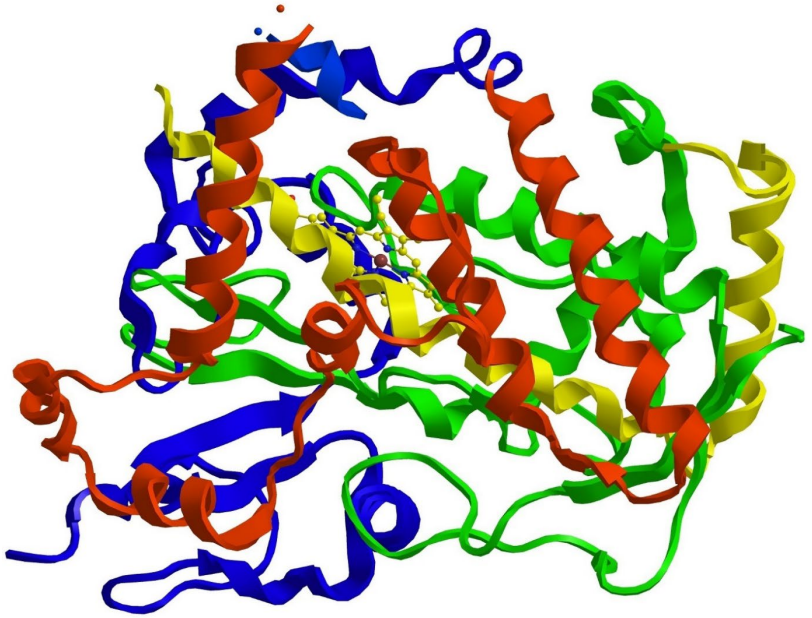
- Use chemical inhibitors specific to CYP enzymes to determine CYP enzymes responsible for clearance
- Typically use metabolism-dependent CYP inhibitors (longer lasting inhibition, better for longer incubation times)

Design:

- 1 or 2 concentrations of test article
- Panel of CYP inhibitors
- 1 concentration HLM
- 1 incubation time point



Reaction Phenotyping: Other Enzymes



Approach may be limited depending on enzymes evaluated

- Is recombinant test system available?
- How specific are chemical inhibitors?

For UGT enzymes:

- Recombinant human UGT enzymes
- Chemical inhibition (limited to UGT1A1, UGT1A4, UGT1A9, UGT2B10)
- Correlation analysis is not recommended due to minimal variability in UGT enzyme activity among individuals in kit.

XT Products (Metabolism/Reaction Phenotyping)

Subcell Fractions:

- Microsomes, S9, and/or Cytosol
- Multiple tissues (liver and extrahepatic)
- Human, Monkey, Rat, Mouse, Dog

Recombinant Enzymes (Distributed):

- Cypex
- Phase I and Phase II enzymes

Primary Hepatocytes

- Pooled donors
- Suspension (2-6 hours)
- Attaching (longer than 6 hours)
- Multiple species

Reaction Phenotyping Kit (Patented)

- Well-characterized individual donor HLMs

Support Reagents

- Rapid Start – NADPH regenerating system
  - For subcellular fractions
- Optimized hepatocyte media
  - Opti: Thaw, Plate, Incubate, and Culture
- Control metabolites (Distributed)
  - Cypex
- Technical Support
  - Recommended Protocols
  - Troubleshooting

Thank you for watching!

Questions? Get in touch through the Contact Us tab on our website

Please contact your regional account manager if you are interested in a placing a reaction phenotyping or other drug metabolism study for your drug's development