Time Dependent Inhibition of P450 Enzymes in Drug Discovery and Development

Technical Aspects
Used in Decision Making
Limitations and Assumptions

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Outline

- Introduction
  - Objectives of the PhRMA DMTG Sponsored Effort on TDI
- Current State of the Science of TDI for Cytochrome P450 Enzymes
- Practical Aspects
  - Conduct of TDI Experiments
    - Drug Development: Determination of K_I and k_{inact}
    - Drug Discovery: Abbreviated Methods of Identifying and Categorizing TDI
  - Prediction of DDI from TDI
- Application of TDI in Drug Development Decision Making and Clinical DDI Study Strategy

Introduction

From Appendix C-2 of the current FDA draft guidance on DDI

1065 4. **Determining Whether an NME is a Mechanism-Based Inhibitor**
1066
1067 Time-dependent inhibition should be examined in standard in vitro screening protocols,
1068 because the phenomenon cannot be predicted with complete confidence from chemical
1069 structure. A 30-minute pre-incubation of a potential inhibitor before the addition of substrate
1070 is recommended. Any time-dependent and concentration-dependent loss of initial product
1071 formation rate indicates mechanism-based inhibition. For compounds containing amines,
1072 metabolic intermediate complex formation can be followed spectroscopically. Detection of
1073 time-dependent inhibition kinetics in vitro indicates follow-up with in vivo studies in
1074 humans.
1075

Introduction

- PhRMA Drug Metabolism Technical Group initiated and sponsored a cross-company working group to assess practices across the industry regarding TDI in December of 2007
- Fifteen scientists engaged in in vitro drug metabolism research volunteered
- Process:
  - Surveyed the industry on current practices (87 questions)
    - Drug development and discovery
    - In vitro techniques
    - Use of data in decision-making
  - Analysis of survey data
  - Development of consensus recommendations
  - Summarized in published white paper (*Drug Metabolism and Disposition* – July 2009)

Today: Share these findings with you

Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

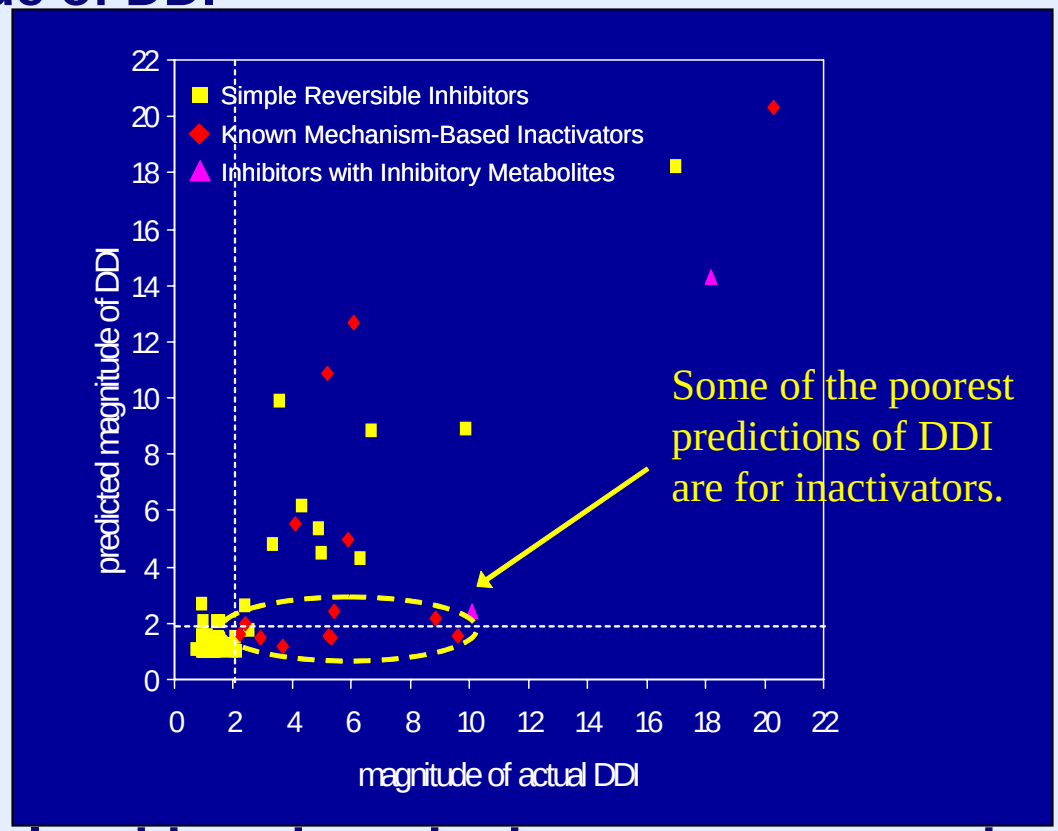
- First: Some Definitions:
- Time-Dependent Inhibition (TDI): A kinetically defined phenomenon in which inhibition increases the longer the inhibitor is incubated with the enzyme
- Mechanism-Based Inactivation (MBI): A mechanistically defined phenomenon in which an inhibitor first serves as a substrate for an enzyme but then inactivates the enzyme
- MBI is a subset of TDI
- Demonstrating that a compound is an MBI requires experiments beyond those merely demonstrating time-dependent inhibition
- In typical drug development and discovery, TDI is frequently shown but MBI is more rarely shown
- TDI is needed for DDI prediction; cannot just rely upon reversible inhibition for DDI prediction
- MBI can help in early drug design; knowing the mechanism informs medicinal chemists on how to remove this property through drug design

Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- TDI for human P450 enzymes is important for DDI
- Some of the most notorious perpetrators of DDI act through TDI
  - Paroxetine and MDMA – CYP2D6
  - Zileuton and Rofecoxib – CYP1A2
  - Gemfibrozil – CYP2C8 (via glucuronide conjugate)
  - TDI for CYP3A4 is common
    - Erythromycin, clarithromycin, troleandomycin
    - Diltiazem
    - Nefazodone
    - Grapefruit (dihydroxybergamottin)
    - Mibefradil - withdrawn

Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

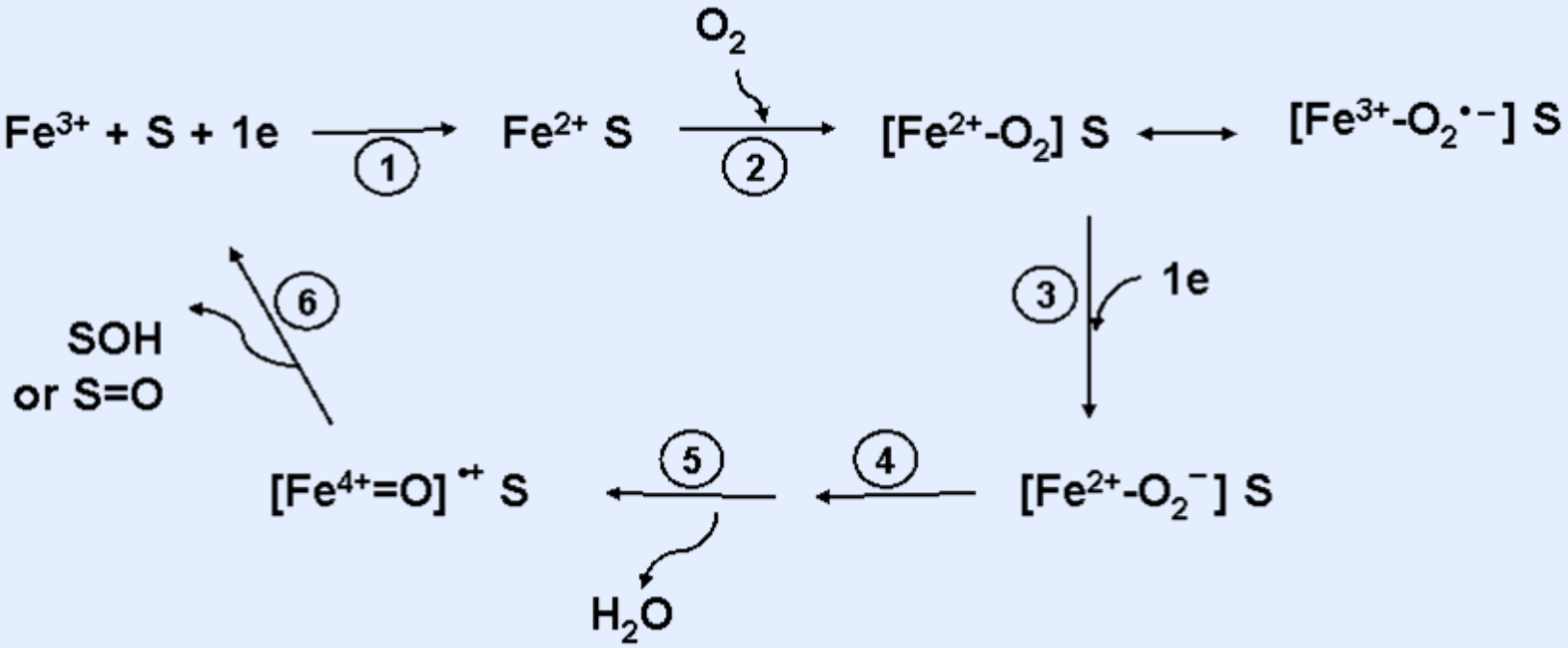
- Reversible inhibition experiments will usually show a TDI to be having an effect on the enzyme, but they will fail to predict the magnitude of DDI



- So properly addressing whether new compounds can be TDI is important

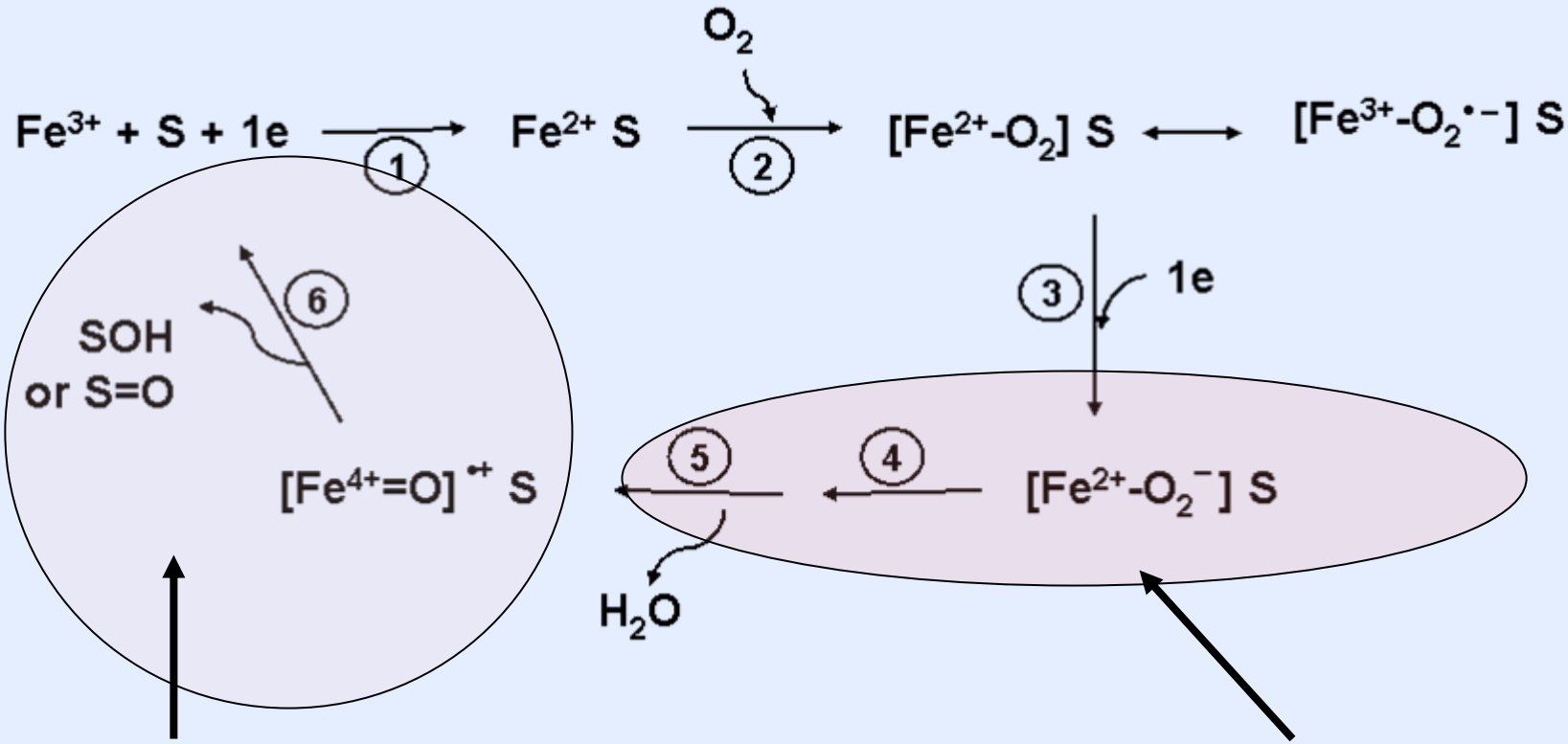
Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- **The P450 Catalytic Cycle**



Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- **The P450 Catalytic Cycle**



Inactivation that is due to MBI happens here

Inactivation that is due to ROS happens here

Relevant for DDI

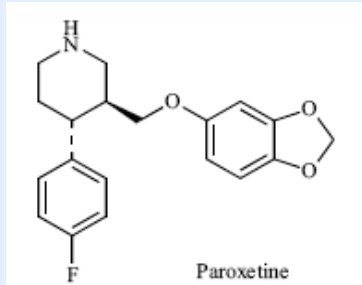
Relevance for DDI unknown

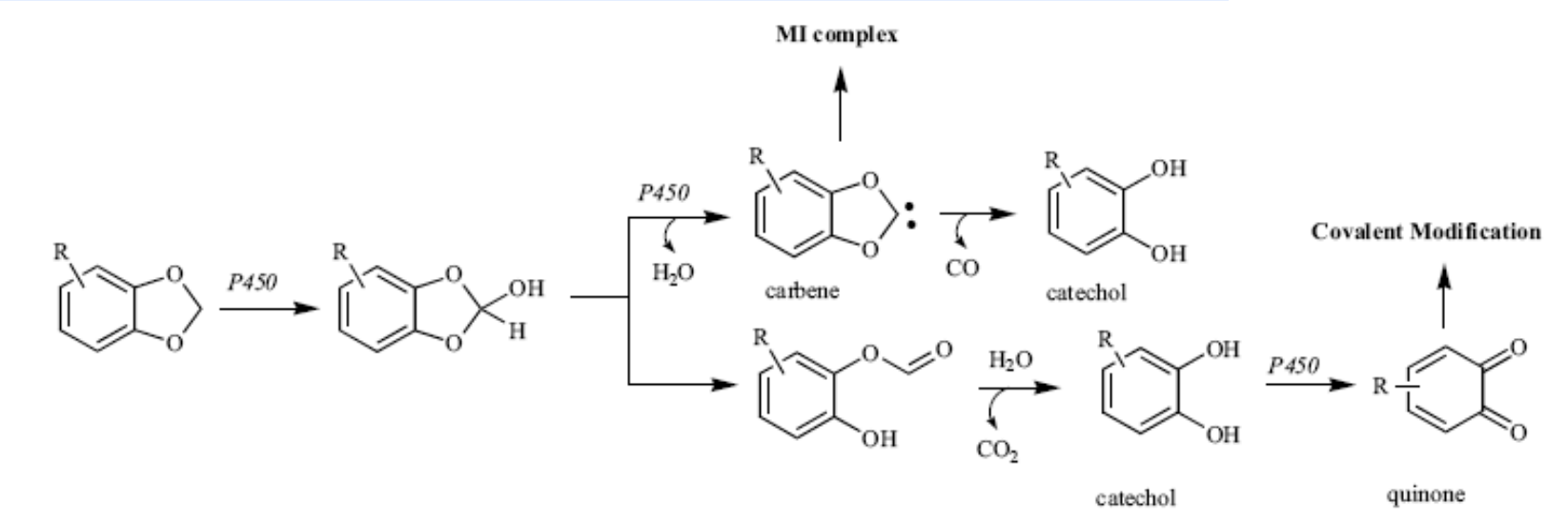
Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- Three Common Mechanisms of P450 MBI:
  - Metabolite-Intermediate Complex Formation
  - Heme Adduct Formation
  - Protein Adduct Formation
- Irrespective of the mechanism, all three are relevant for DDI

Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

- Metabolite-Intermediate Complex Formation
- Also referred to as quasi-irreversible inactivation because there are conditions in vitro that can be applied to sometimes reverse the inactivation
- Example: paroxetine

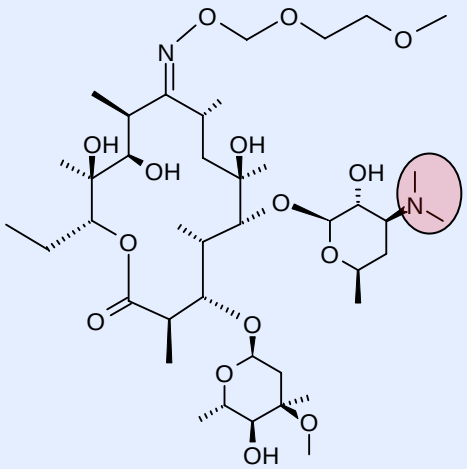




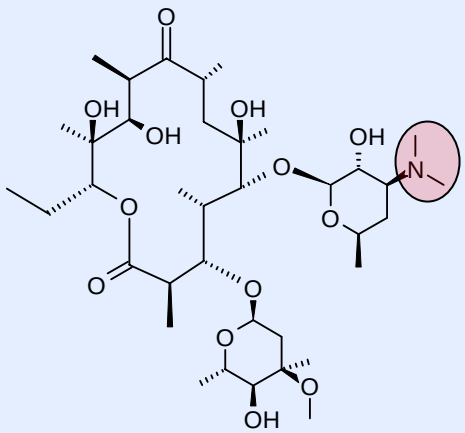
- MI complexes can be observed spectrally

Time-Dependent Inhibition of P450 Enzymes: Current State of the Science

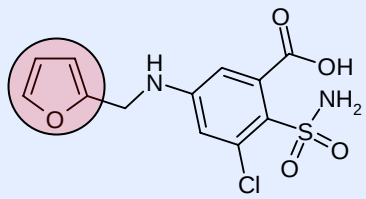
- There is some SAR developed for P450 inactivation
  - Several functional groups have been identified that are capabile of doing this
  - But P450 TDI is not predictable from structure alone



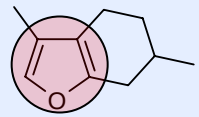
roxithromycin



erythromycin



furosemide



menthofuran

Practical Aspects: The Conduct of TDI Experiments

Practical Aspects: The Conduct of TDI Experiments

- Compared to typical reversible inhibition experiments, TDI experiments are much more complex, and challenging to convert to high throughput techniques
- Three methodologies
  - "Dilution" method – very commonly used
  - "Two-Step" method – less commonly used
  - "Progress Curve" method – rarely used

Practical Aspects: The Conduct of TDI Experiments

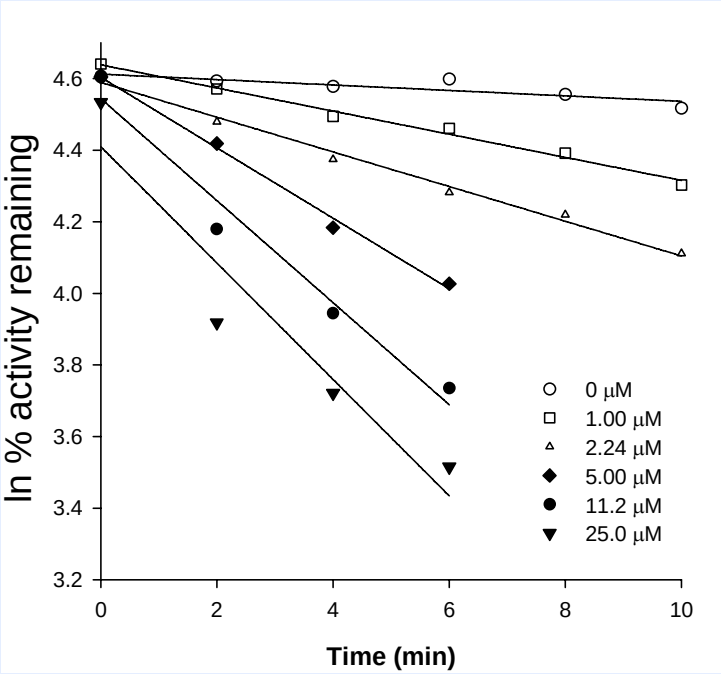
- The dilution method:
  - Two parts
    - Test compound incubated with enzyme source and NADPH (“inactivation” incubation or “preincubation”)
    - At various time points, aliquots of the inactivation incubation mixture are diluted into a second incubation containing saturating substrate and NADPH (“activity” incubation)

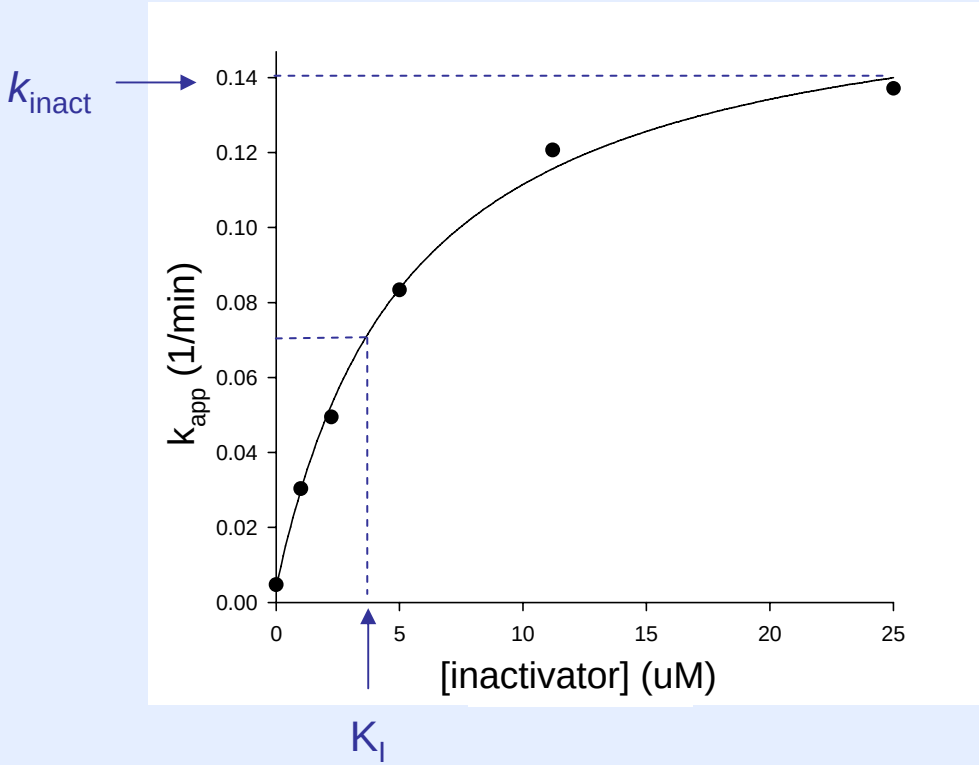
Practical Aspects: The Conduct of TDI Experiments

- The two-step method
  - Two parts
    - Test compound incubated with enzyme source and NADPH
    - At various time points during the incubation, saturating substrate is added and incubated for a set time
  - Disadvantage that inactivation can occur during the substrate activity assay
- Progress Curve method
  - Inactivator, substrate, enzyme source, and NADPH are all incubated together
  - Product is measured at several time points
  - Rate of decline in activity is compared to vehicle control (no inactivator)
  - This approach may be more realistic to in vivo, but its capability to be used to predict DDI is not established

Practical Aspects: The Conduct of TDI Experiments

- **Back to the dilution method…**
- **The output data should look like this:**

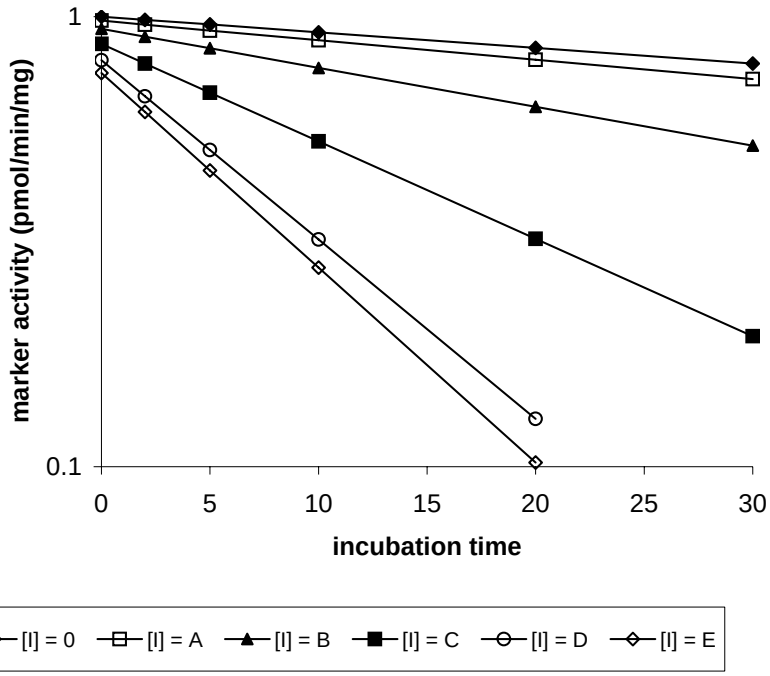


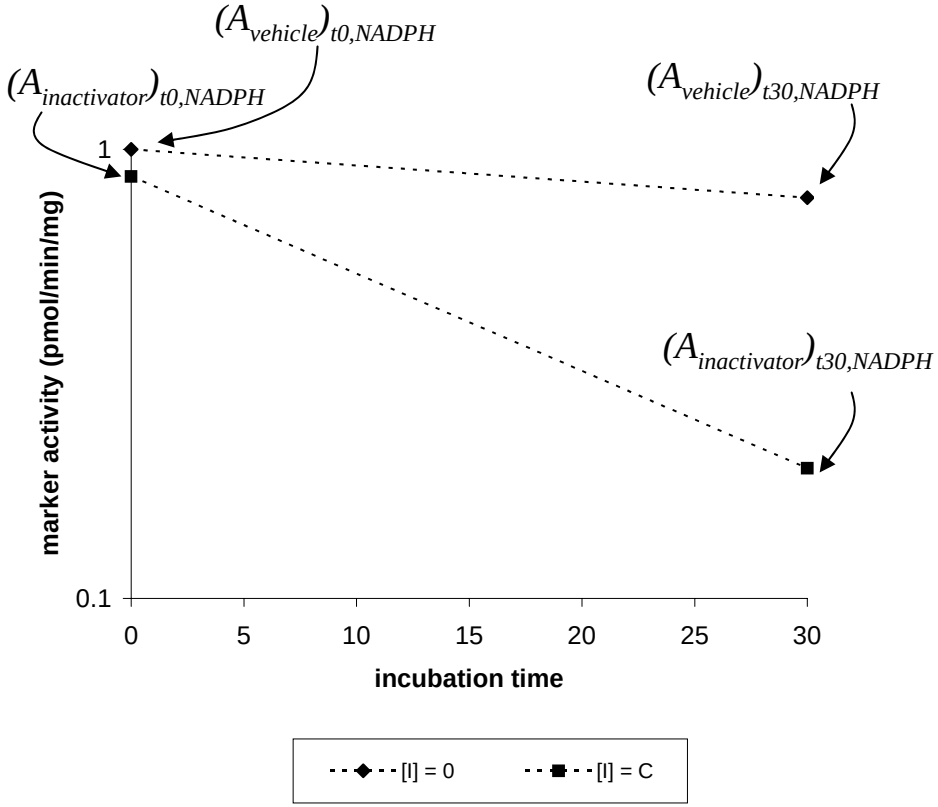


Practical Aspects: The Conduct of TDI Experiments

- The determination of k_{inact} and K_I is appropriate for compounds in drug development, but far too involved to use for hundreds of compounds encountered in a drug discovery program.
- Abbreviated methods have been developed to establish whether a new compound is a TDI or not

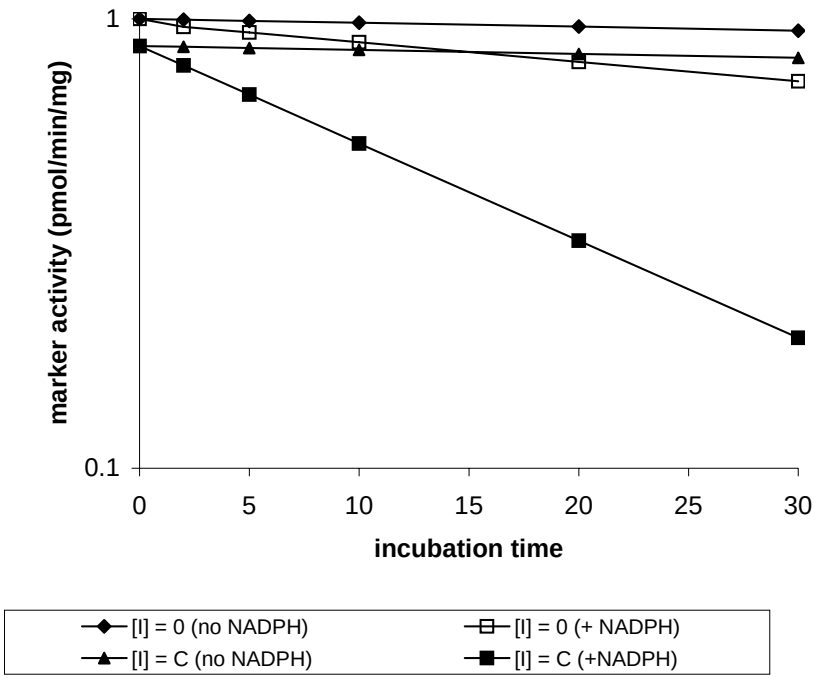
Practical Aspects: The Conduct of TDI Experiments

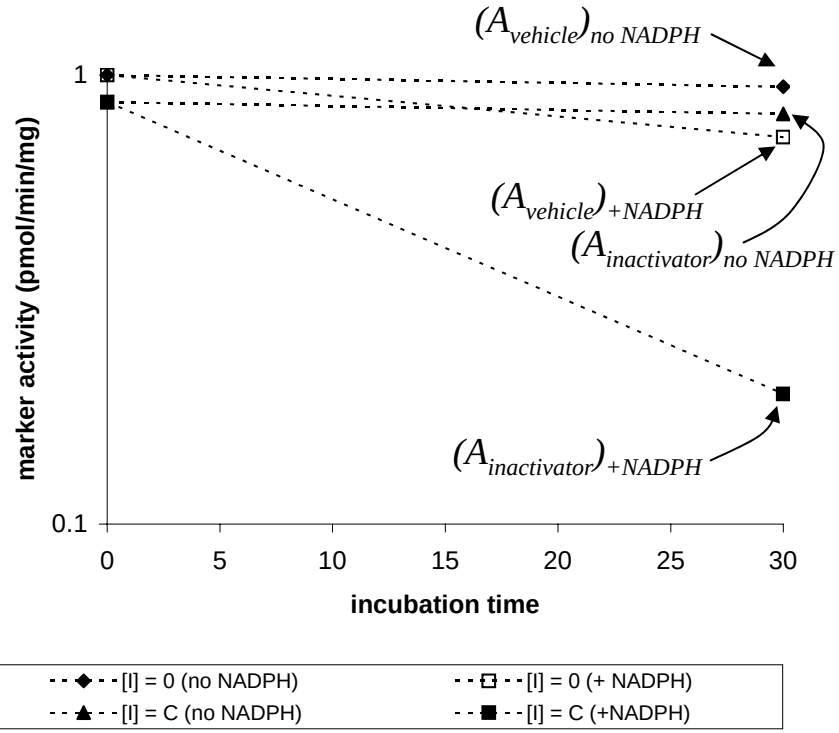




$$\%\ \text{activity loss} = 100 \bullet \left[\left(\frac{A_{inactivator}}{A_{vehicle}} \right)_{t_0\ NADPH} - \left(\frac{A_{inactivator}}{A_{vehicle}} \right)_{t_{min} NADPH} \right]$$

Practical Aspects: The Conduct of TDI Experiments





$$\%\ \text{activity loss} = 100 \bullet \left[\left(\frac{A_{inactivator}}{A_{vehicle}} \right)_{no\ NADPH} - \left(\frac{A_{inactivator}}{A_{vehicle}} \right)_{+NADPH} \right]$$

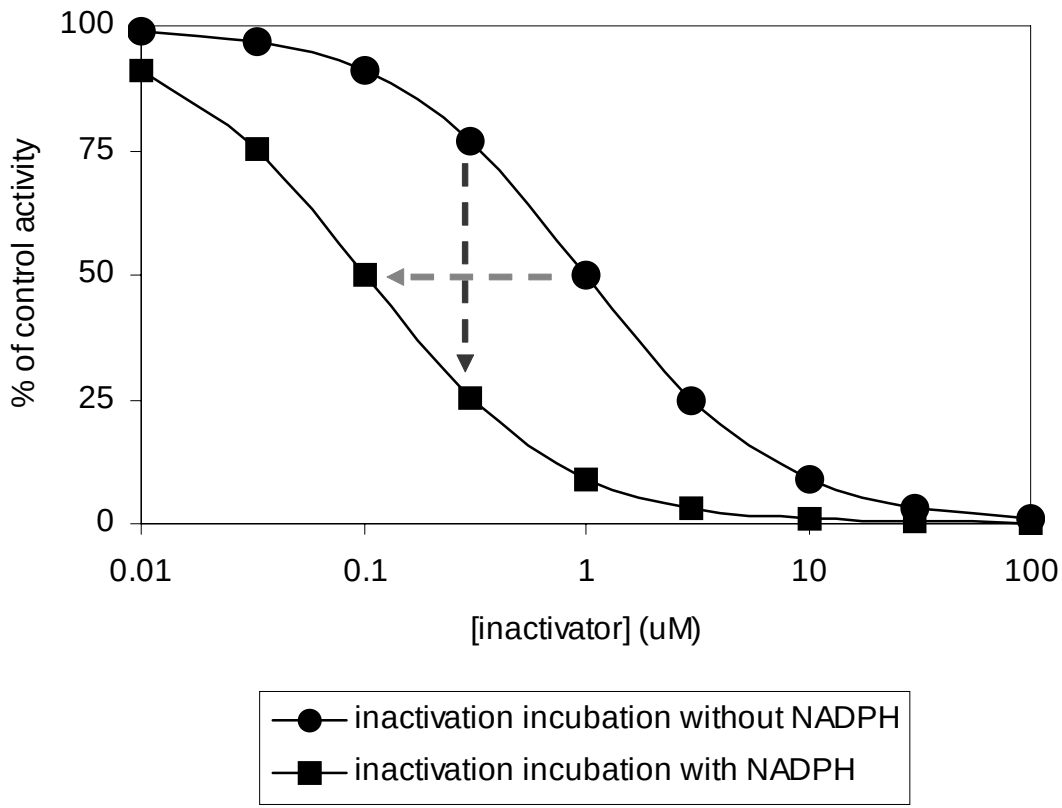
Figure 2

Practical Aspects: The Conduct of TDI Experiments

- These abbreviated methods can be used to identify those compounds requiring determination of K_I and k_{inact}
- If changes of 20-25% or less are observed in 30 min with pooled HLM, then the compound is not considered a concern for DDI caused by TDI

Practical Aspects: The Conduct of TDI Experiments

- IC_{50} shift experiment: Another abbreviated experimental design to identify TDI
- Run as a typical IC_{50} experiment in the 'control' state
- Compared to an IC_{50} determined after the test compound has been preincubated with enzyme and NADPH for 30 min
- If IC_{50} difference is 1.5X or more, the compound is an inactivator



Practical Aspects: Predicting DDI from In Vitro TDI

- **Mathematical Model**
  - **First published by Mayhew, et al., 2000**
  - **Fundamental equation:**

$$\frac{AUC_i}{AUC} = \frac{1}{\left(\frac{k_{\text{deg}}}{k_{\text{deg}} + \frac{[\text{I}] \times k_{inact}}{[\text{I}] + \text{K}_\text{I}}} \right)}$$

[I] = ***in vivo*** **inactivator concentration**
k_{deg} = ***in vivo*** **degradation rate constant for the inactivated enzyme**
K_I **and** k_{inact} = **determined** ***in vitro***

Practical Aspects: Predicting DDI from In Vitro TDI

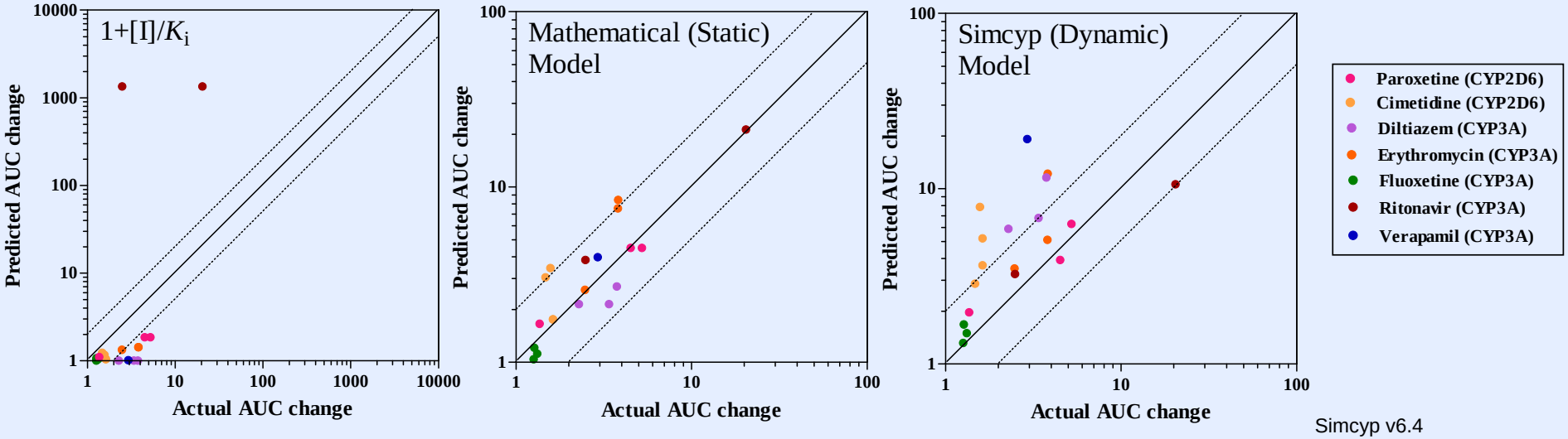
- **Mathematical Model**
  - **Built in important terms: fraction of the victim drug cleared by the affected enzyme and the contribution of the intestine (for CYP3A)**

$$\frac{AUC_i}{AUC} = \frac{1}{\left(\frac{f_{m,CYP}}{1+\left[\frac{k_{inact}/k_{deg}}{1+\left[K_I/[I]\right]}\right]}\right)+\left(1-f_{m,CYP}\right)} \times \frac{1}{F_g+\left(1-F_g\right)\times\left(1+\left[\frac{k_{inact}/k_{deg,CYP3A}}{1+\left[K_I/[I]_g\right]}\right]\right)}$$

[I] = ***in vivo*** **inactivator concentration**
k_{deg} = ***in vivo*** **degradation rate constant for the inactivated enzyme**
K_I and k_{inact} = **determined *in vitro***
F_g = **fraction of the victim drug that evades intestinal extraction in the uninhibited condition**
$f_{m,CYP}$ = **fraction of the victim drug cleared by the affected enzyme**

Practical Aspects: Predicting DDI from In Vitro TDI

- **Predictions of actual change in AUC due to TDI using different approaches**
  - 21 clinical trials involving TDI; data extracted from Einolf (2007)
  - It is well known that the [I]/K_i approach can not be used for TDI
  - Other models, such as the above described Mathematical Model and models that incorporate time-varying [I], offer better assessments of risk for TDI



Simcyp v6.4

Practical Aspects: Predicting DDI from In Vitro TDI

- **Uncertainties in Mathematical Model**
  - **[I] : free or total? circulating or hepatic?**
  - **k_{deg}: what are the true in vivo values?**
  - **How well established are in vivo F_g and $f_{m,CYP}$ for various probe substrates? (e.g. midazolam)**

We'll return to this question in a little while…….

Practical Aspects: Predicting DDI from In Vitro TDI

- Simulation and Modeling of DDI Caused by TDI
  - In general, the underlying mathematics are the same and the same uncertainties in input parameters exist
  - Permits more sensitivity testing of input parameters
    - *e.g.:* If one assumes that the in vitro data are x-fold inaccurate, what is the impact on the predicted DDI?
  - Permits inter-individual variability to be assessed with population simulation
  - Assists with clinical DDI trial design *e.g.* frequency of dosing, number of doses, wash-out duration, etc.

Summary of the PhRMA Survey of TDI Practices

Summary of the PhRMA Survey of TDI Practices

- Survey of 87 questions
- Covered strategic and technical aspects, as well as how TDI data are used for prediction of DDI
- Solicited feedback from 32 PhRMA companies; received 17 anonymized responses
- Overall conclusion: Far more agreement than disagreement

Summary of the PhRMA Survey of TDI Practices

- On strategic aspects of TDI:

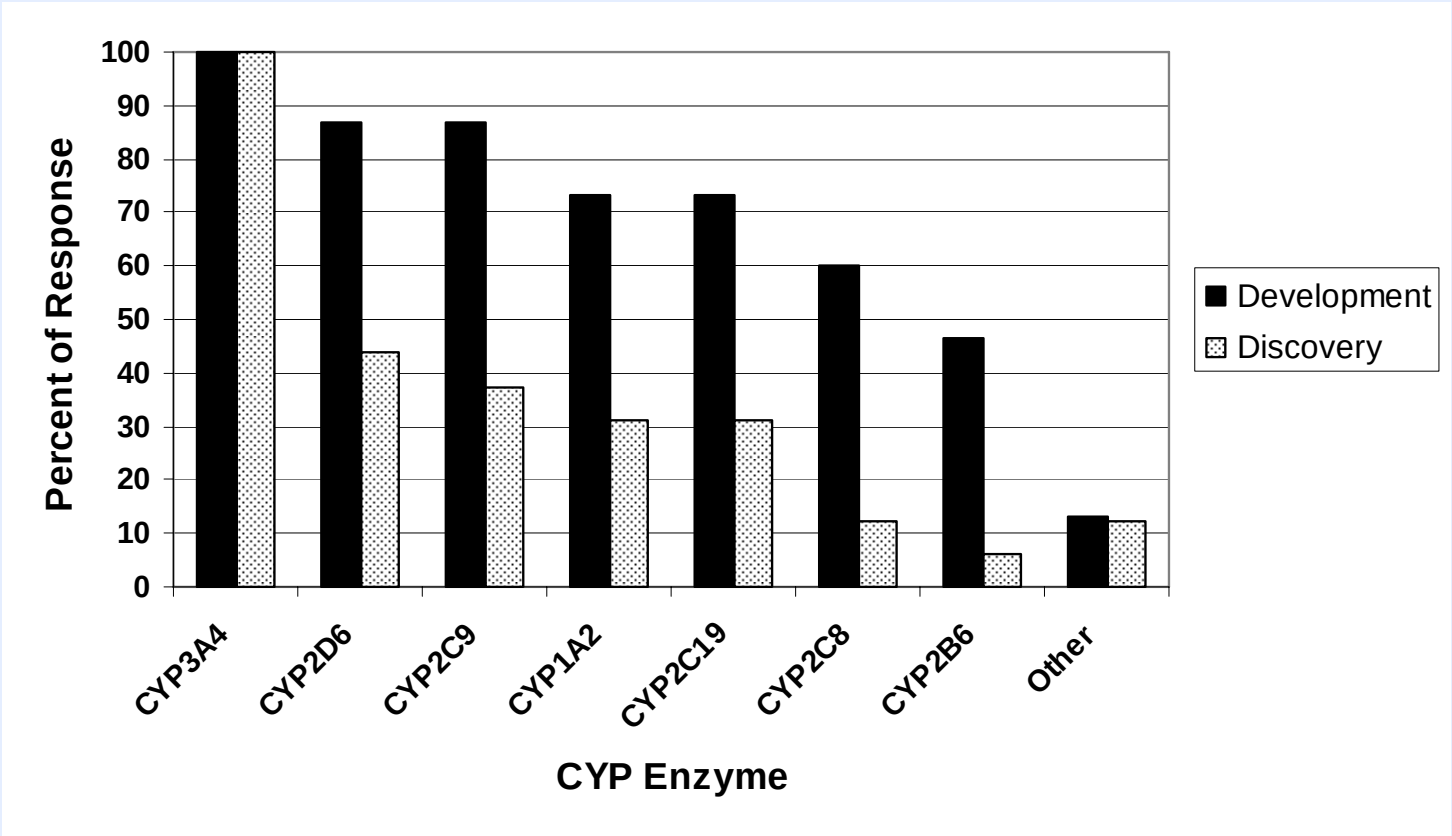
Common Practices	Divergent Practices
All assess TDI during drug discovery/development continuum TDI data are used for predicting DDI	Timing of definitive assays for clinical DDI predictions ranges from lead optimization through phase 1 No common cut-off values for TDI data for further progression of NMEs Use of various study designs for TDI assessment in drug discovery (e.g., IC_{50} shift vs % activity loss at single NME concentration, etc.) No common consideration of structural alerts

Summary of the PhRMA Survey of TDI Practices

- On technical aspects of TDI:

Common Practices	Divergent Practices
Pooled human liver microsomes (100%) The same major P450 enzymes tested LC-MS/MS for measurement of probe substrates (100%) Solvent control at each time point (- test article + NADPH) are used (100%) Determine the log-linear phase of enzyme inactivation (100%) Conduct control incubations without NADPH Replicate determinations of K_I and k_{inact} are conducted Positive controls are included Test article depletion not measured	Fold dilution used during IC_{50} shift determinations range from no dilution to greater than 10-fold Number of NME concentrations used to determine inactivation parameters (6 or greater) Number of time-points used (4 to >6) Data Analysis Log-linear regression (k_{obs}) followed by non-linear fitting to determine K_I and k_{inact} parameters Reciprocal plot (e.g., Kitz-Wilson) to estimate K_I and k_{inact} Global non-linear regression

Summary of the PhRMA Survey of TDI Practices



Summary of the PhRMA Survey of TDI Practices

- On using the data to predict DDI:

Common Practices	Divergent Practices
Current models cannot accurately predict DDI due to TDI Existing models can categorize compounds as weak, moderate or potent clinical DDI risks DDI predictions to decide whether to conduct a DDI study and inform its design	Various models (static vs. dynamic, inclusion of gut first-pass vs. no gut first pass etc.) are used for predicting DDI risk based on K_I and k_{inact} values. Various values used as surrogates for $[I]_{in\ vivo}$ (e.g. C_{max}, free vs total, etc) Microsomal and plasma protein binding corrections used by some

Summary of the PhRMA Survey of TDI Practices

- **Overall: Convergence of technical aspects of study conduct**
- **Problem Areas: uncertainty in precise predictions of DDI, mostly due to uncertainties regarding input parameters (or parameters embedded in computer models)**
  - **$[I]_{in\ vivo}$ – free vs total; systemic vs estimated hepatic**
  - **k_{deg} for P450 enzymes (no way to directly measure)**

Enzyme	Range of $t_{1/2}$ values (hr)	
	Estimated from In Vitro Data	Estimated from In Vivo Data
CYP1A2	36-51	39-105
CYP2B6	32	no data
CYP2C8	23	no data
CYP2C9	104	no data
CYP2C19	26	no data
CYP2D6	70	51
CYP2E1	27	50-60
CYP3A4	26-79	36-140

Summary of the PhRMA Survey of TDI Practices

- Recommendations and Agreements
  - TDI is important for drug discovery and development
  - Use a two-tiered strategy:
    - Abbreviated method to identify TDI
    - Determine K_I and k_{inact} for those compounds that are positive in the abbreviated method (e.g. change in inhibition of 20-25% at a single [I] or 1.5X difference in shifted IC_{50})
  - Mechanistic experiments to determine MBI are not necessary; TDI is good enough
  - Always check CYP3A, due to its importance

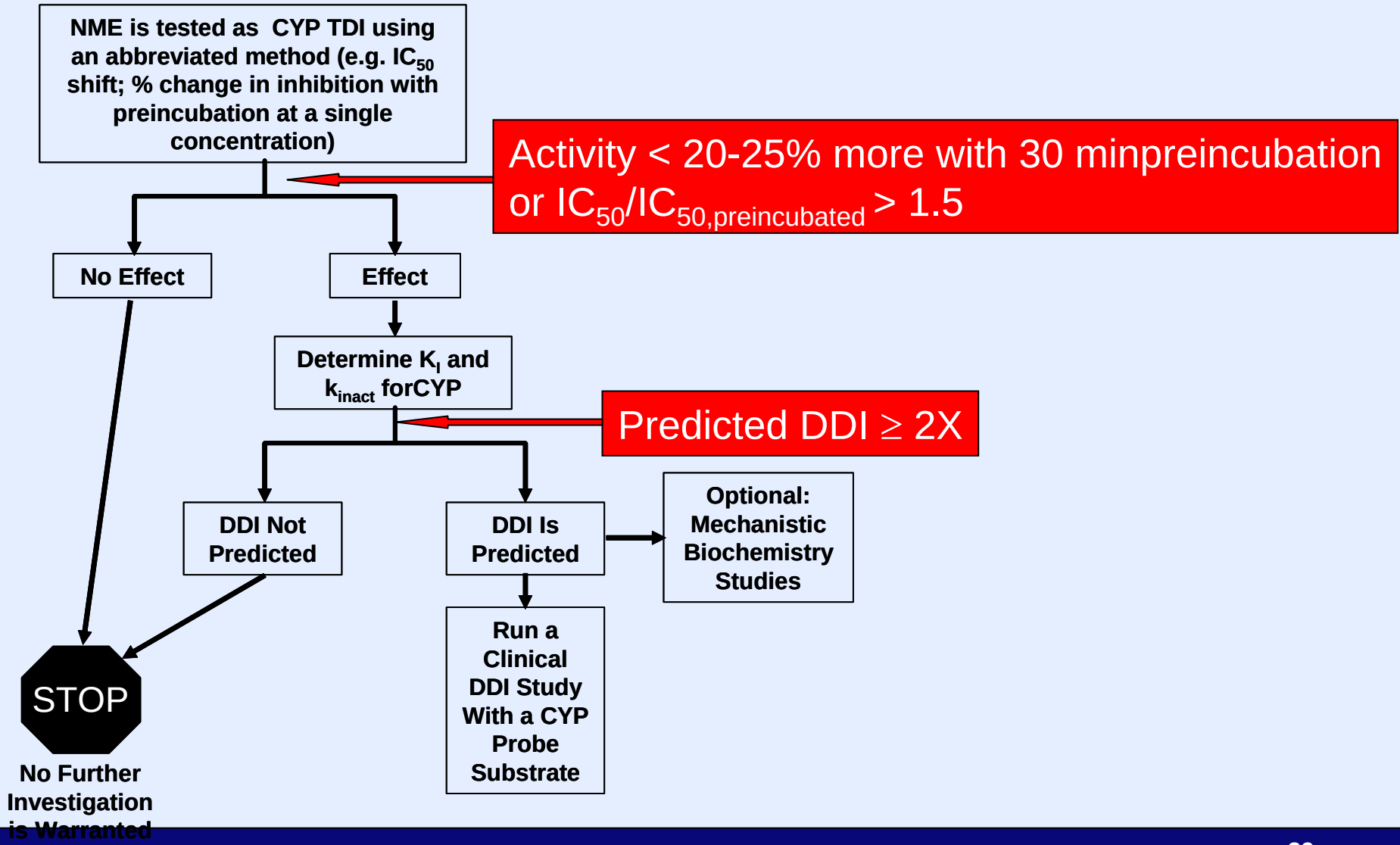
Summary of the PhRMA Survey of TDI Practices

- Recommendations and Agreements
  - Dilution approach to measurement of TDI (10X dilution)
  - Pooled HLM as the source of enzyme
  - Saturating [S] for K_I-k_{inact} determinations
  - Use 5 or more [I]; should flank K_I

Summary of the PhRMA Survey of TDI Practices

- Recommendations and Agreements
  - Predicted DDI of 2X or more is important; most likely do an in vivo study
  - Because of remaining uncertainty in certain input parameters for DDI prediction, each lab should verify that DDI can be predicted for known positive control inactivators using their prediction method, input parameters, and their own in vitro TDI data
  - This area of science will evolve and will need revisitation in the future

TDI and DDI Decision Tree



Placement of Assessments of TDI in the Drug Discovery and Development Timeline

Very Early: TDI of CYP3A4 as a candidate selection criterion

Early: Identification of TDI for major human P450 enzymes; identification of possible DDI issues

In Development: Determination of TDI; Prediction of DDI

In Development: Conduct of DDI Studies as Needed

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