

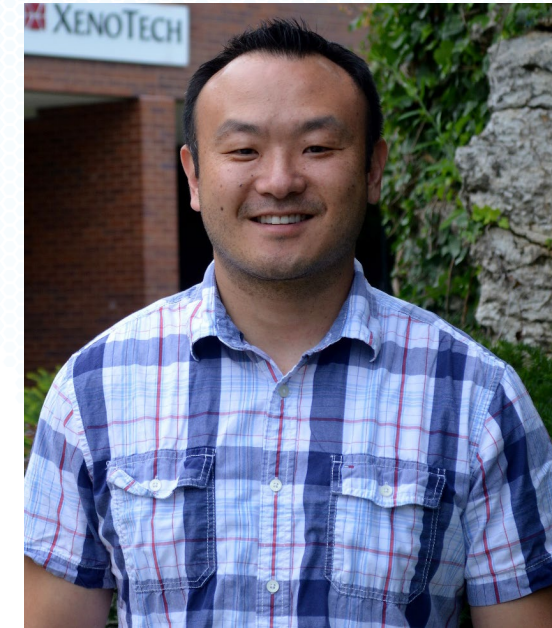
Drug Metabolism Studies: **Stability**



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Overview

- Test systems for stability studies
- Stability study endpoints
- Example data
- Client considerations
- Products and test systems for stability studies



Test systems for stability studies

Cryopreserved hepatocytes are the most commonly used test system.

- Intact hepatocytes contain the major hepatic drug-metabolizing enzymes required to study the four categories of xenobiotic biotransformation:
 - Hydrolysis
 - Reduction
 - Oxidation
 - Conjugation
- Other options:
 - Microsomes or S9 (need appropriate cofactors, NADPH/UDPGA)
 - Attachable (plated) cryopreserved hepatocytes



Stability study endpoints



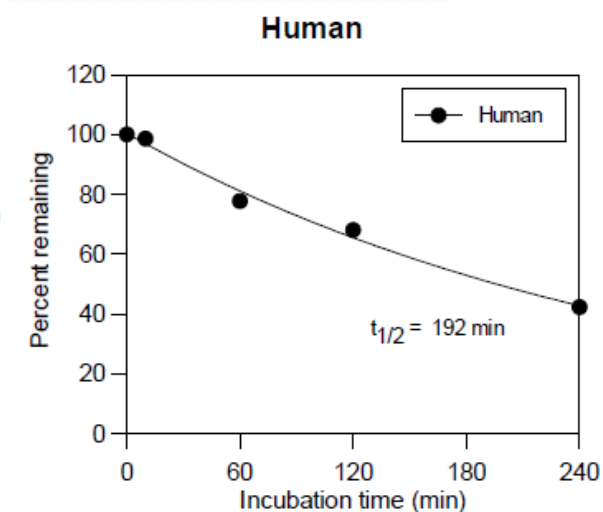
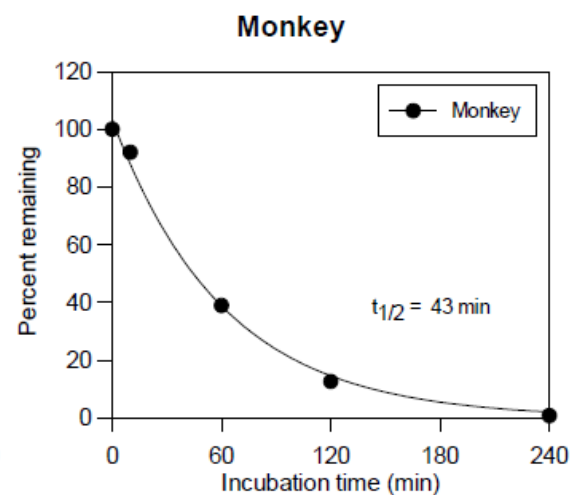
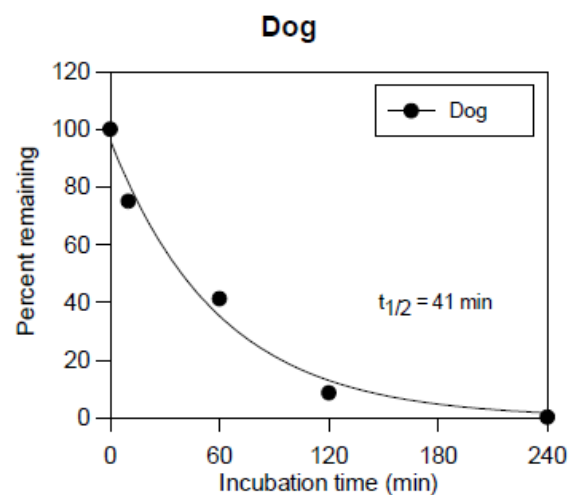
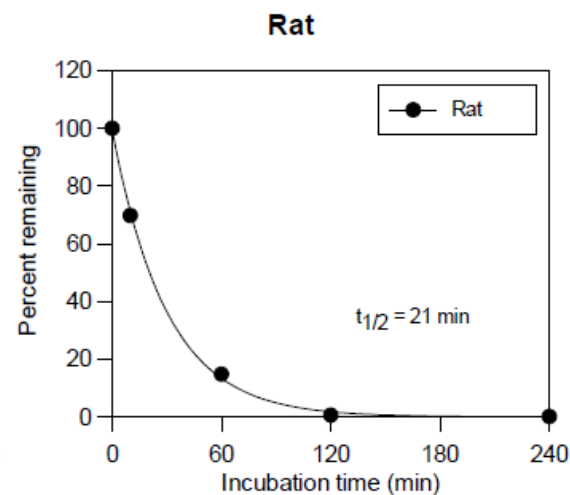
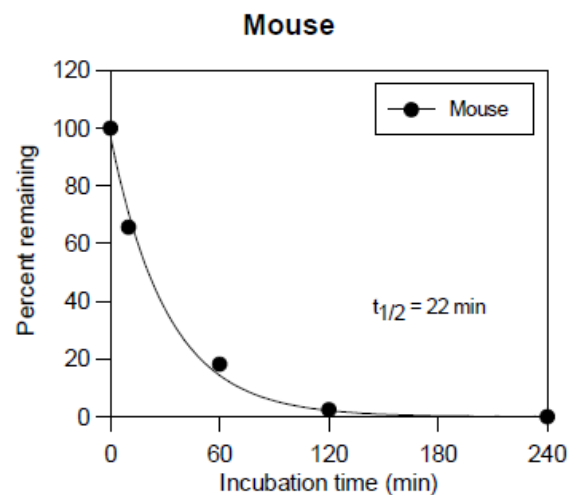
- Test compound loss
- Metabolite formation
- Both

Metabolic Stability

- Metabolic stability influences oral bioavailability and plasma half-life of a compound
- Various test systems and species can be used, depending on questions to be answered
 - Hepatocytes, suspended or plated
 - Microsomes
 - S9
 - Cytosol
 - Others
- Multiple species (mouse, rat, dog, monkey, human...and others)
- Multiple time points (zero + three)
 - Allows for $t_{1/2}$ and estimate of intrinsic clearance
- Want to keep [TA] low
- Look at substrate loss (or metabolite formation)
- Marker substrate positive control (e.g., 7-ethoxycoumarin)
- Data summary



Metabolic stability example results



Considerations for the client

- Test article considerations (MW, molecule type, solubility issues, etc.)
- The test article structure, MW
- Any known or suspected metabolite reference standards?
- Any known relevant in-vitro data (e.g., reaction phenotyping)?
- Analytical method, if available (LC-MS/MS)?





Drug Metabolism Test Systems

Pooled primary human hepatocytes

Suspension assays

Human or animal model

Pool size:

10-20 donor

HepatoSure (100 donor)

Tissue-specific subcellular fractions

Human and small animal

Liver fractions

Pooled microsomes most common (also S9, cytosol)

Pool size (HLMs):

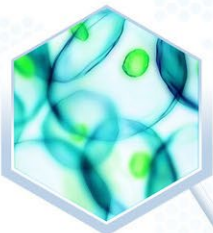
50 donor pool

Xtreme 200

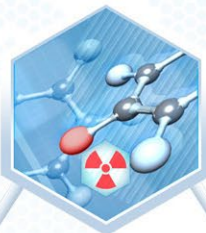
Extrahepatic

Dependent on route of admin. or exposure

Cell & Tissue-Based Products



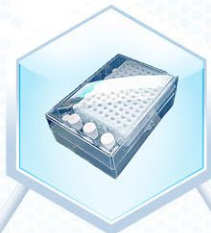
Radiolabeling



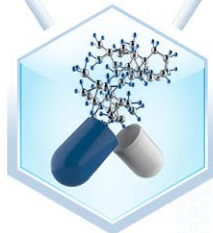
in vitro ADMET & Pharmacology



Metabolite ID & Production



Screening



API Manufacturing



in vivo ADMET & QWBA



Bioanalytical

Thank you!