HOW DO I SELECT THE RIGHT SPECIES FOR MY TOXICOLOGY PROGRAM?

Presented by:

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Meet Our Presenters

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Conducting Nonclinical Toxicology Studies

- **How do you know which species to use?**
  - Six commonly used species
  - Each have their own concerns
  - Growing concerns around use of NHPs
  - Recently concerns over the use of dogs
- **Selection of a species for determining the safety profile of your program should be based on scientific rationale**






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How do I Know Which Species Is Right for My Program?

- **The universal answer of a toxicologist – it depends**
  - Guidance and test article type
    - ICH S6R1 - biologics
    - ICH M3 (R2) – small molecules
  - Dose route
    - Dermal indications

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Biologics

- **Based on pharmacology**
  - Does the test article produce the desired pharmacological effect
  - Tiered approach
    - Conservation of target sequence
    - *In vitro* receptor binding/engagement
  - Primarily involves NHP
    - Can involve rodent as well

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Small Molecules

- **Still a major class of test articles in development**
- **Many programs are conducted using rat and dog**
- **How do companies know they are using the correct species?**
  - Are efforts to reduce/eliminate the use of NHPs or dogs
  - There is a scientific approach to justifying the species
- **For small molecules it is all about metabolism**
  - *In vitro* approaches using microsomes or hepatocytes
  - Look at human, mouse, rat, dog, minipig, and NHP
  - Use the rodent and nonrodent that cover human

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Small molecules

- **What if the profiles are comparable?**
  - Pharmacology
  - Sensitivity
  - Exposure
- **Bottom line is the selection of the species to be used should be based on science**

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Small Molecules

- **Selection sounds straightforward but how exactly is metabolic profiling conducted**
  - Are microsomes better than hepatocytes?
  - Do you need all the species?
  - How exactly is metabolic profiling accomplished?

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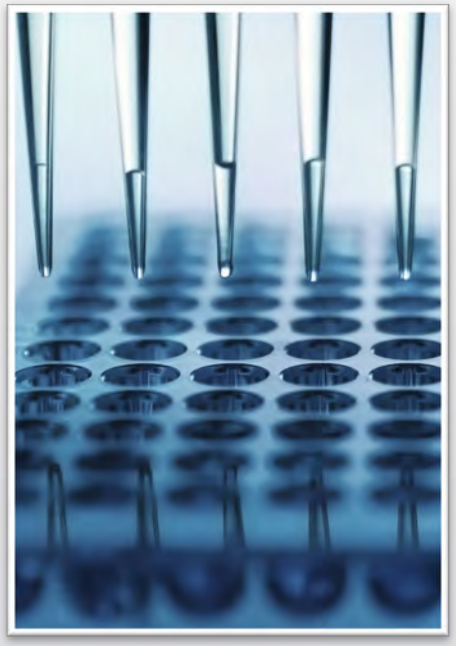
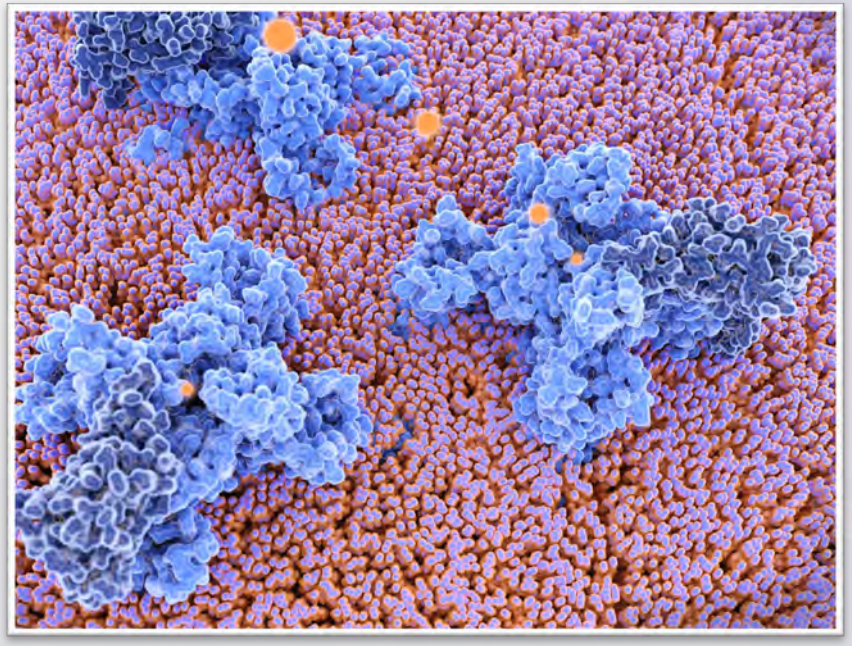
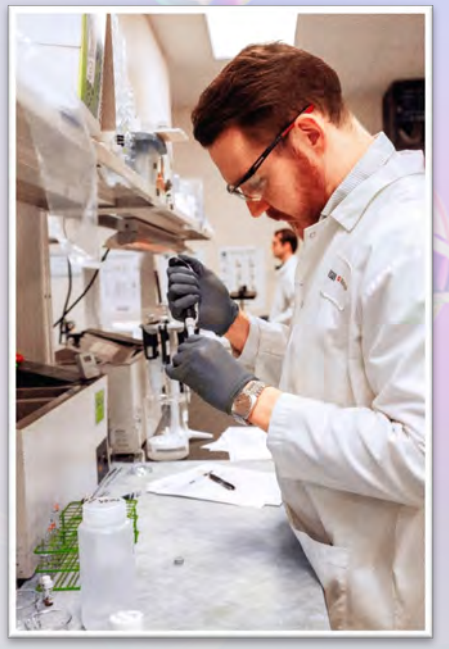
IN VITRO DRUG METABOLISM STUDIES FOR SPECIES SELECTION







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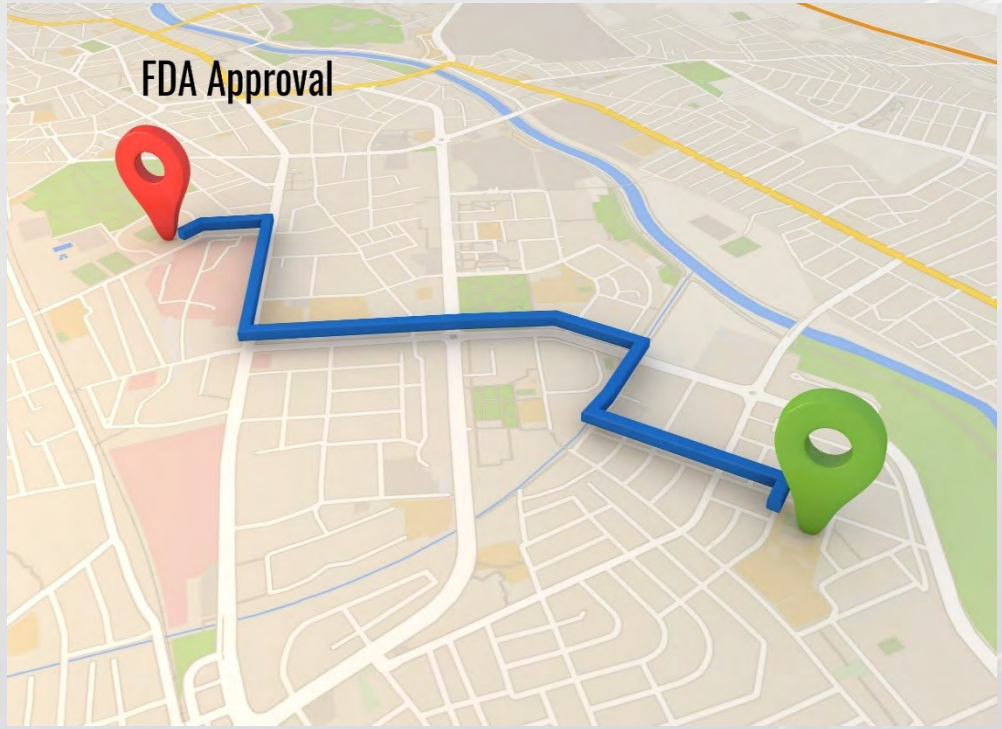
Overview

- Test systems for in vitro studies
- What is available?
- Advantages of each
- In vitro study types
- Stability
- Metabolite characterization
- Enzyme induction



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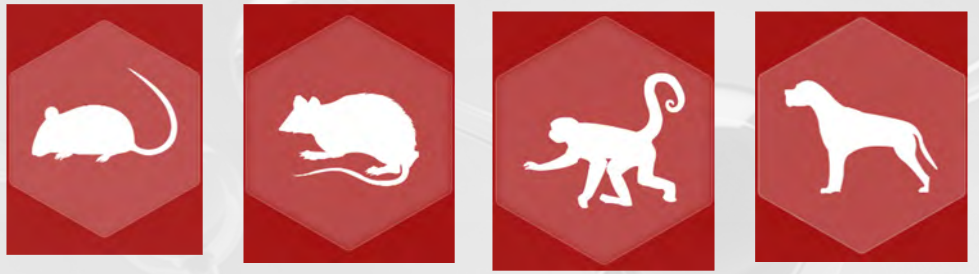
Test systems for *in vitro* studies

Hepatocytes

- Suspension
- Attaching or pre-plated

Subcellular fractions

- Various tissue (liver, lung, skin, etc.)
- Microsomes
- S9
- Cytosol
- Other (lysosomes, tritosomes)



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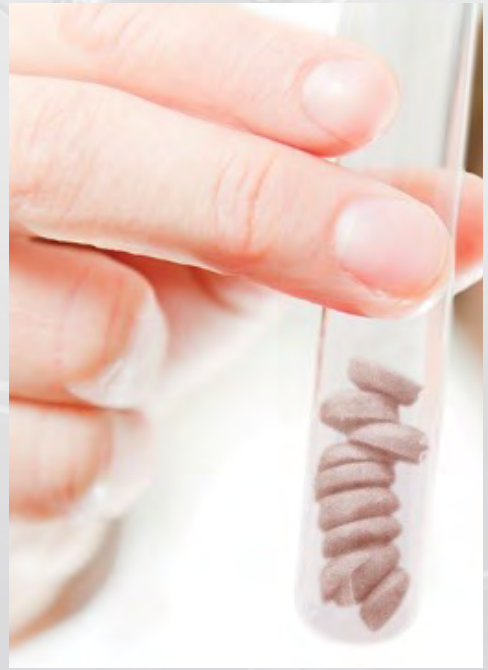
Hepatocytes

Suspended (4-6 hr incubation)

- Metabolic stability
- Metabolite characterization
- Medium Throughput Screening (MTS)

Attaching/plated (> 5 d incubation)

- Enzyme induction
- Metabolic stability
- Metabolite characterization
- Low turnover compounds



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Non-human Hepatocytes

- Pooled from clinically-relevant species
- Long-term lot availability and reproducibility
- High enzyme activity levels
- Consistent lot-to-lot activity levels
- Used for in vitro drug metabolism studies
- Characterized using LC/MS/MS methods

Animal Species

- **Dog** – Beagle
- **Guinea Pig** – Hartley Albino
- **Hamster** – Golden Syrian
- **Minipig** – Gottingen
- **Monkey** – Cynomolgus
- **Mouse** – IGS CD-1
- **Rabbit** – New Zealand White
- **Rat** – IGS Sprague-Dawley, Wistar & Wistar Han

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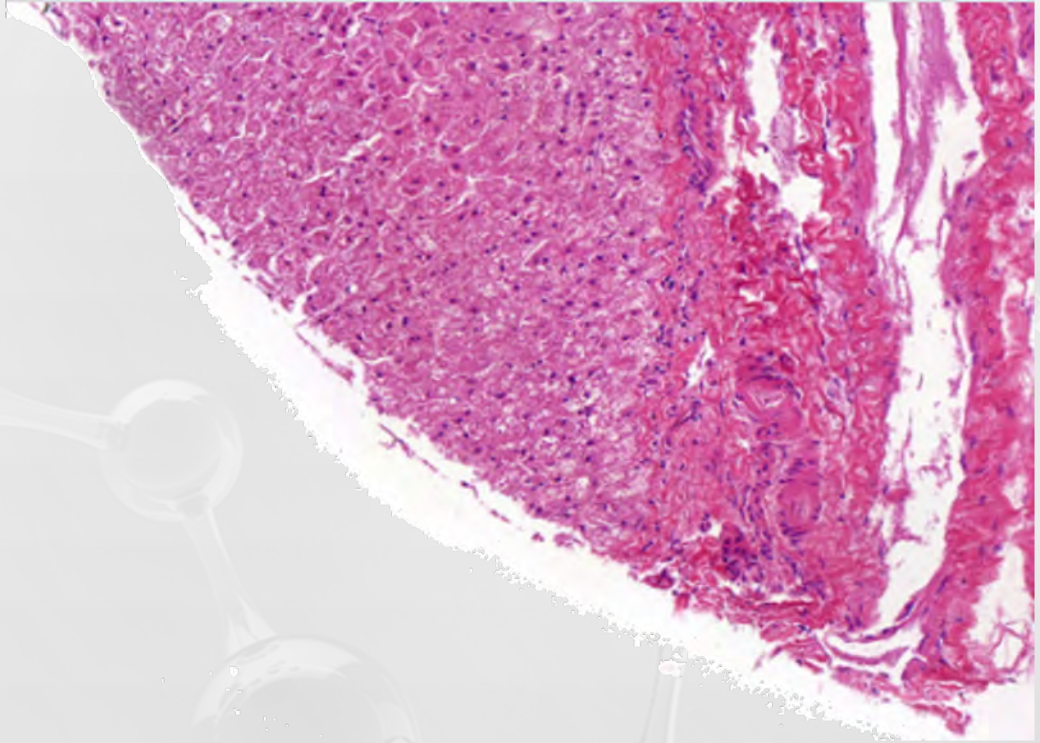
Subcellular Fractions



Many fractions from many tissues & species

- Microsomes
- S9
- Cytosol
- Liver, lung, skin, kidney, intestine, etc.
- Mouse, rat, mini-pig, dog, rabbit, guinea pig, hamster, monkey

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Subcellular Fractions *(CONTINUED)*

Co-factors required for many enzymes

- E.g., NADPH for CYP, UDPGA for UGT
- Enzymes can be turned on/off by addition/omission of required co-factors

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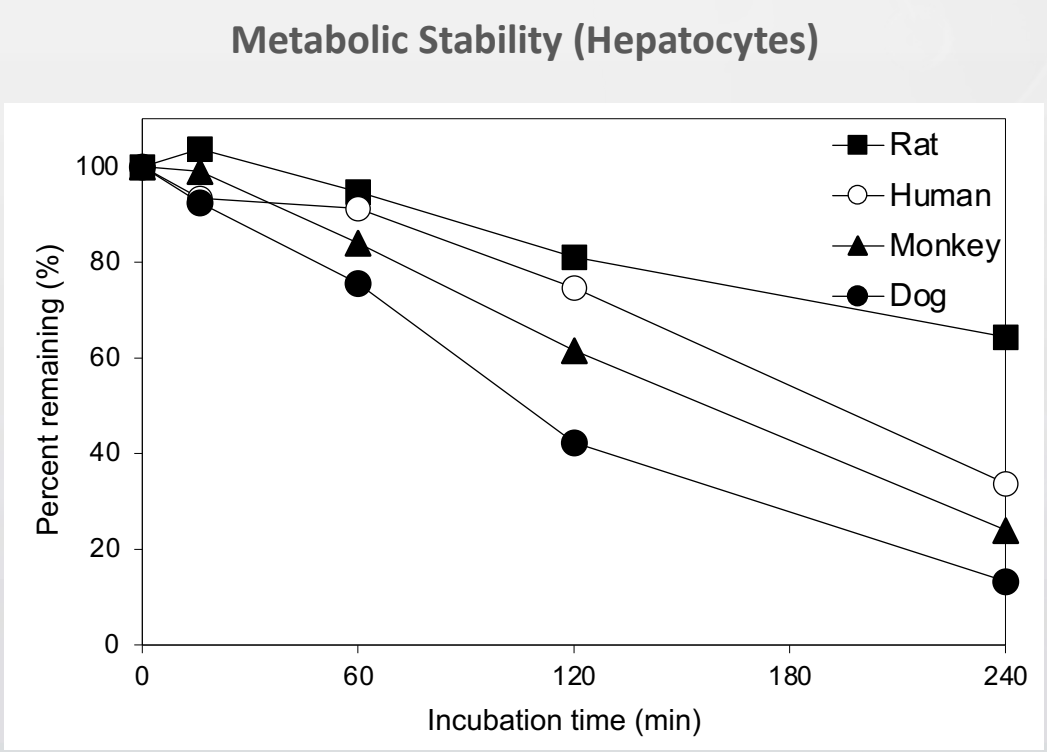
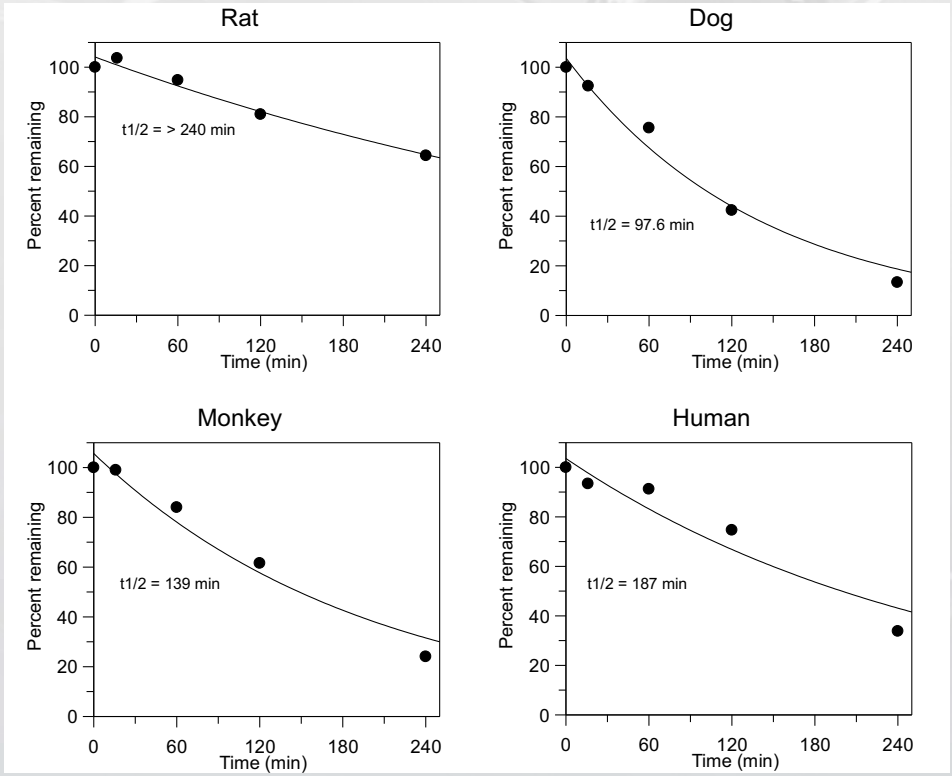
Drug Metabolism: Inter-Species Comparative Metabolism

- **Design: Drug incubations with hepatocytes or subcellular fractions from various species**
- **Typical species: Human, Rat, Mouse, Dog, Rabbit, Monkey, Pig**





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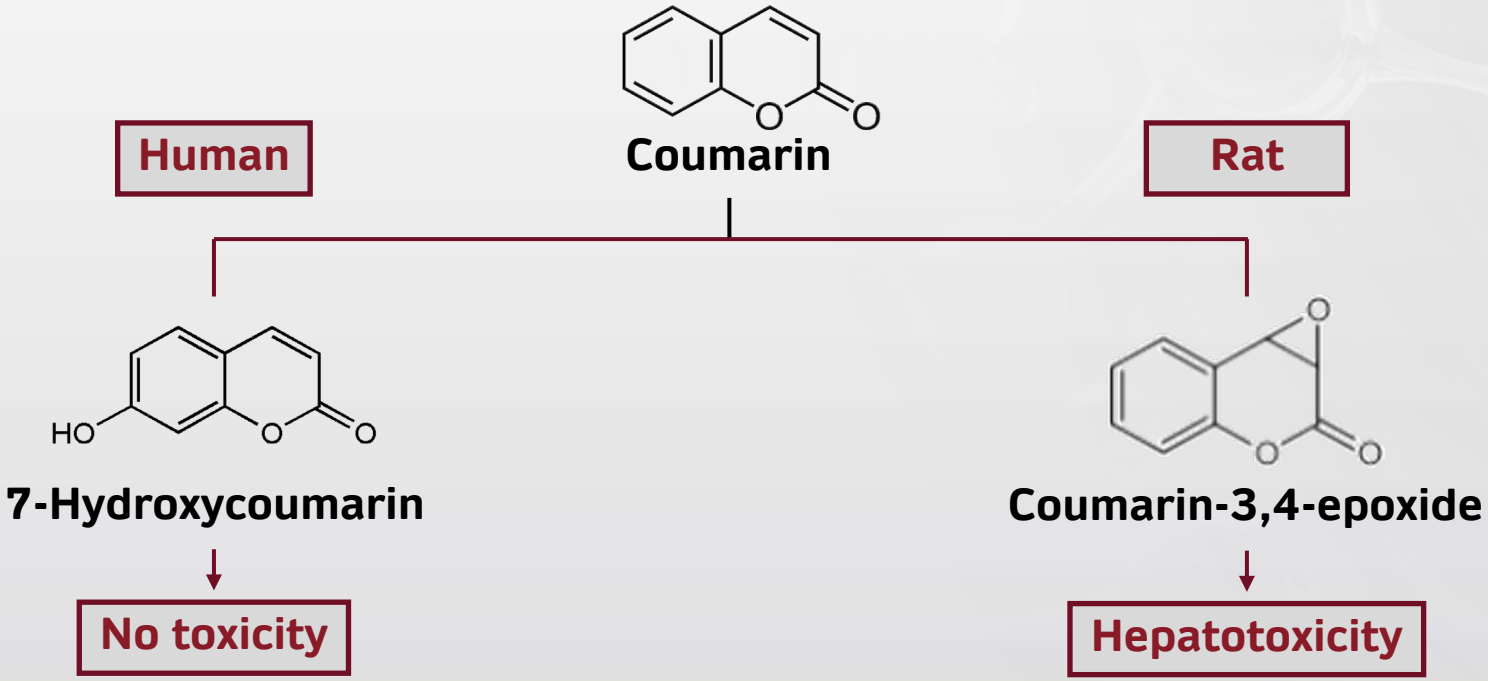
Drug Metabolism: Inter-Species Comparative Metabolite ID



Goals:

- Complete profile of metabolites
- Are there human specific metabolites?
- Which other species have a similar metabolic profile?

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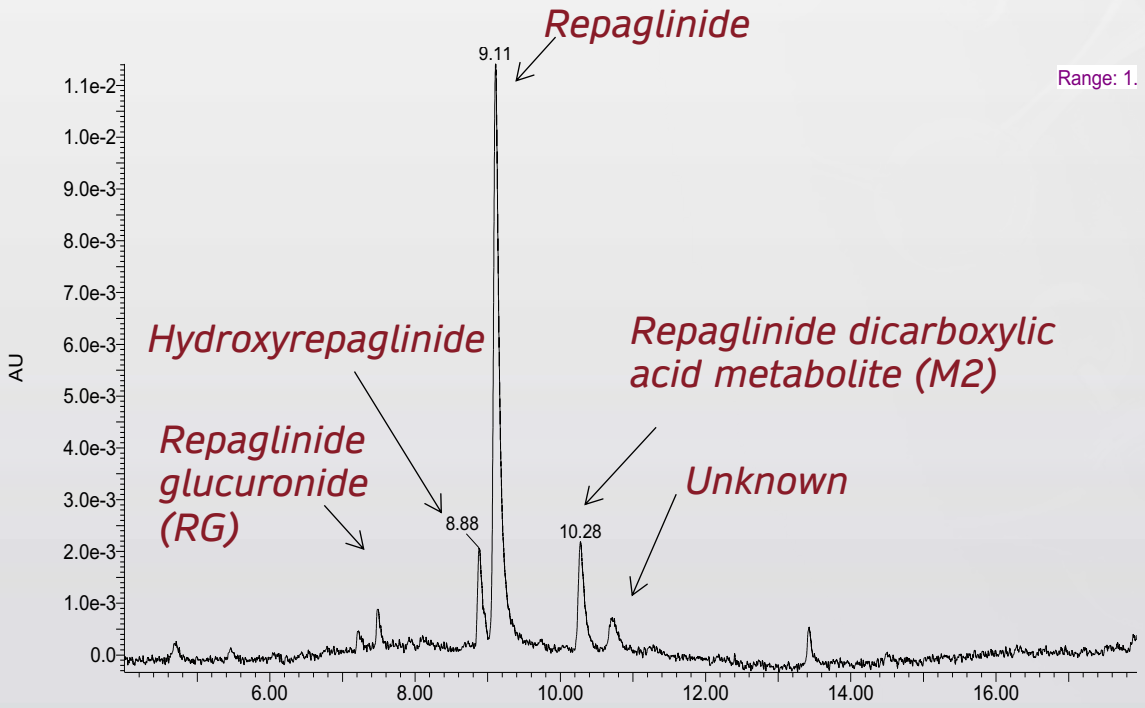
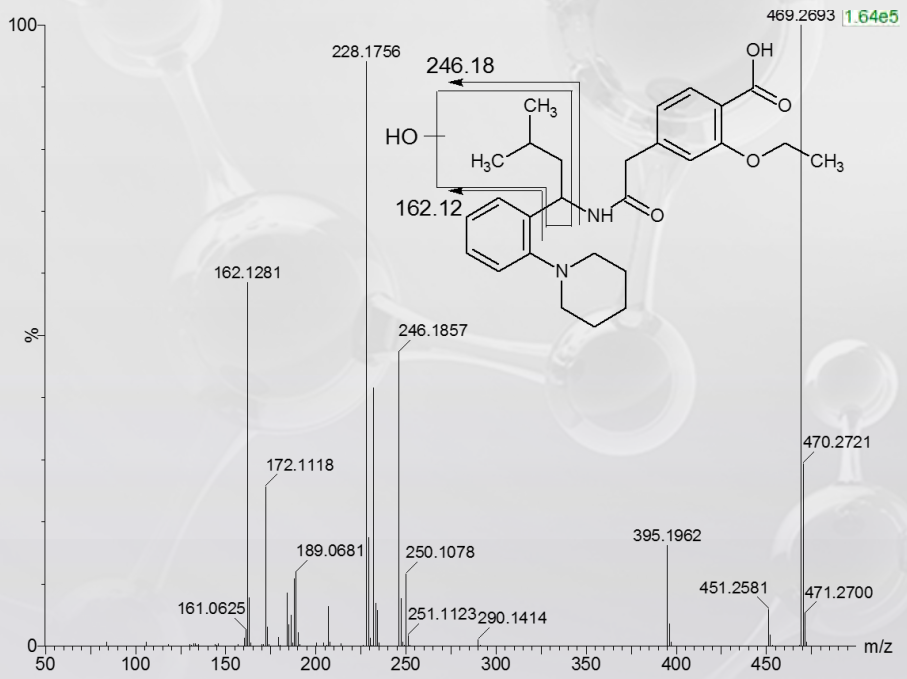
Metabolite ID: LC-MS/MS analysis – Qualitative identification of the metabolites

50 mM Repaglinide; Human hepatocytes; 60 minutes; 37°C



Hydroxyrepaglinide LC-MS/MS



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Cross-Species Met ID:

Component	Retention time (min)	Mass shift	Proposed biotransformation	Mouse	Rat	Dog	Pig	Human
C1	3.43	255.9889	Sulfation + glucuronidation	+	+	+	+	+
C2	3.63	354.0783	Di-glucuronidation + hydrogenation	+	+	+	+	+
C3	3.78	159.9135	Di-sulfation	+	+	+	+	+
C4	4.00	258.0045	Sulfation + glucuronidation + hydrogenation	+	+	+	+	+
C5	4.41	161.9298	Di-sulfation + hydrogenation	+	+	+	+	+
C6	4.44	194.0428	Glucuronidation + oxygenation + hydrogenation	ND	ND	ND	+	+

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Enzyme Induction: In vitro

In vitro induction

- Induction examined in cryopreserved, characterized attaching hepatocytes
- Plated hepatocytes treated with TA for 3 days
- Only requires small amounts of TA (mg amounts)
- Mouse, rat, dog, monkey
- mRNA or enzyme activity endpoints

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Enzyme Induction: Ex vivo

Ex vivo induction

- Inducted in laboratory animals
- Mouse, rat, dog, monkey
- Typically done following tox studies
- Animals dosed by sponsor and liver/tissue samples sent out
- Larger amounts of TA (depending on tox species)
- mRNA or enzyme activity endpoints

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“Why conduct these studies? Is this just box checking?”

No. The information in aggregate has real utility:

1. **Provide deeper understanding of the molecule**
   - Metabolism, enzymes involved in metabolism, etc.
   - The information generated from DDI studies goes on the drug label
   - From the pharma company’s perspective these studies can inform go/no-go decisions for a drug candidate
   - Predictive toxicology and dose selection for certain non-clinical *in vivo* studies
2. **Prepare for clinical studies**
   - Prediction of FIH dose and DDI risk
3. **Satisfy regulatory expectations and comply with regulatory guidance**

These studies may appear deceptively simple – maximizing insight can be complex!

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Summary

- Species selection based on scientific reasoning
- Pharmacological based
- Choosing the right test system is important
  - Tox species for *in vivo*
  - Hepatocytes or subcellular for *in vitro*
- In vitro species comparisons can be useful
  - Compound stability
  - Metabolite characterization
  - Enzyme induction (in vivo follow up as needed)
- **All studies are more than just box checking!**
  The information has real utility!

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