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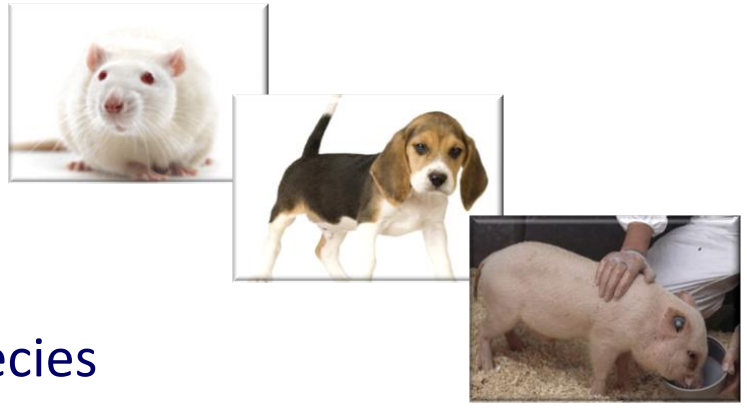
# USE OF MINIPIG IN THE PRECLINICAL DEVELOPMENT OF A NEW PHARMACEUTICAL PRODUCT: METABOLISM AND SELECTION OF THE RELEVANT SPECIES


**S. Cinelli**
*Associate Scientific Director & Head, Business Unit*
*"Genetic, in vitro Toxicology & Immunology"*

**F. Pastori**
*Expert in Mass Spectrometry*

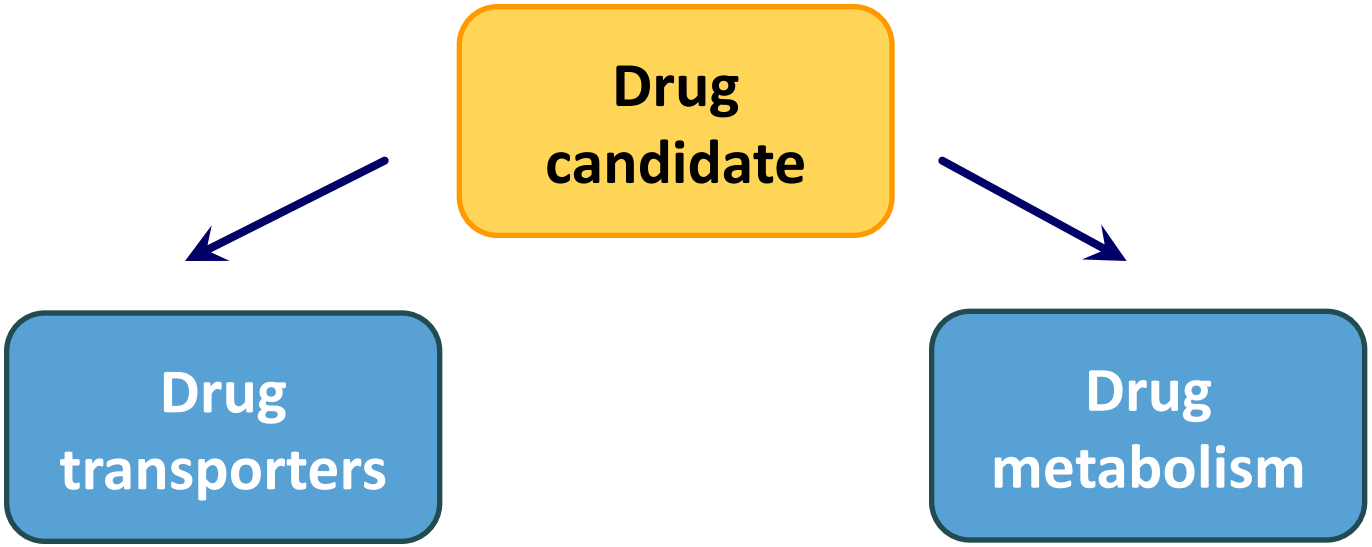
**G. Oberto**
*Scientific Director*

# Selection of non rodent species



## Science based approach

- Pharmacological activity
- Pharmacokinetic profile in different species
- Other species-specific limitations
- Biopharmaceuticals: *in vitro* PD studies and TCR (monoclonal antibodies)



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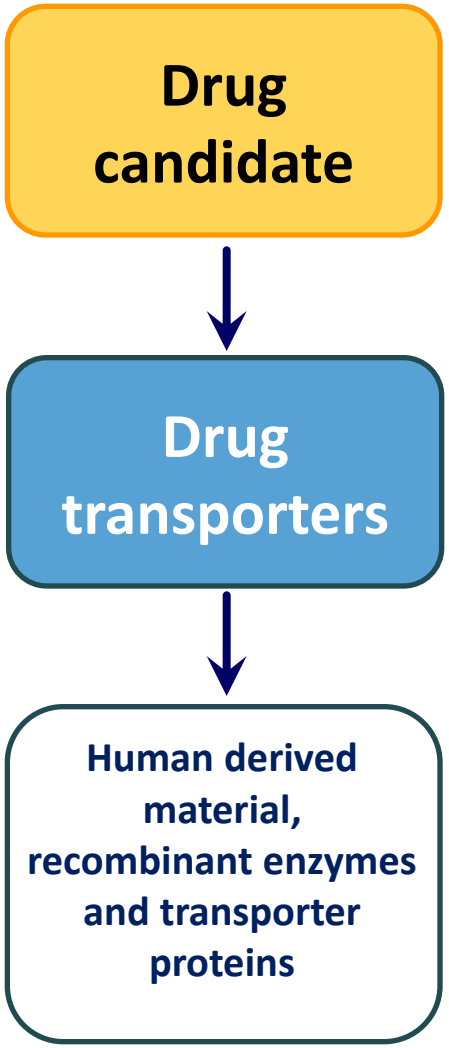


Membrane transporters have emerged as a very important area of interest.

Multidrug resistance in cancer therapy for example can be ascribed to the induction of permeability glycoprotein Pgp resulting in poor exposure to the drug in the cells.

# Selection of non rodent species

**Drug candidate**

↓

**Drug metabolism**

↓

***In vitro* methods**
**Metabolite profiles**

To find the best animal model for human metabolism of a drug candidate, it is highly recommended to study the *in vitro* metabolism in the species concerned

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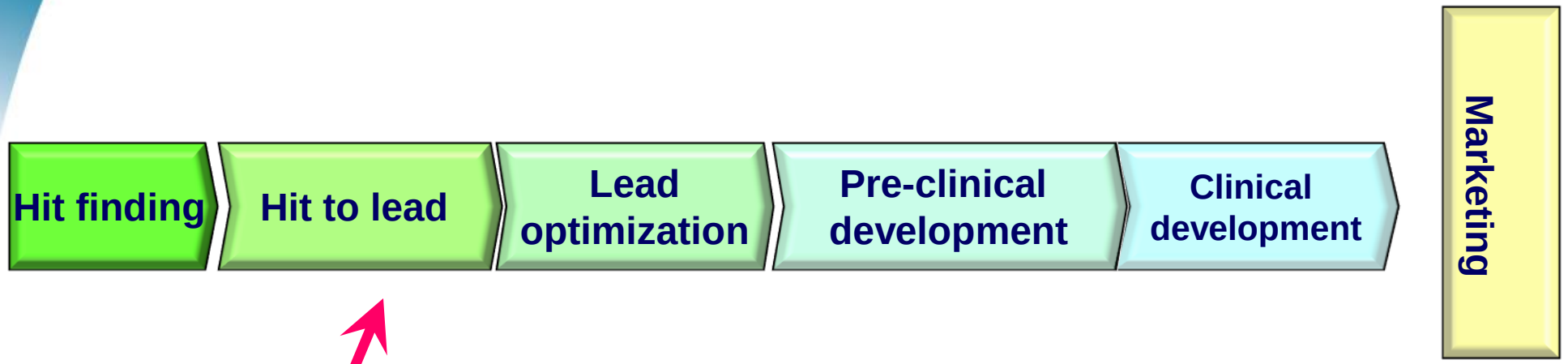


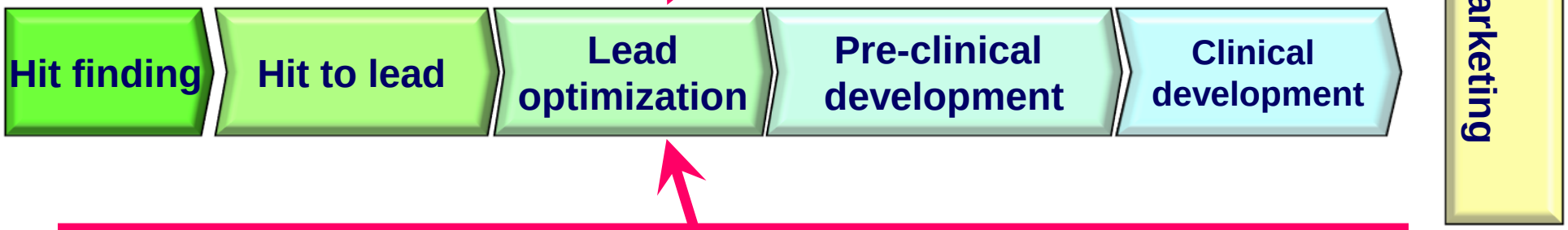

During H2L phase, hits and lead compounds are tested *in vitro* with regard to metabolic stability in human liver cells or liver cell fractions

# Selection of non rodent species

CYP3A4 enzyme can comprise as much as 60% of total CYP in human liver. This form has been shown to be responsible for metabolism of the majority of drugs tested.

During the lead optimization phase, the induction of CYP3A4 enzyme should be evaluated

Induction of CYP3A4 and the resulting increase of metabolism of the drug, render the drug less bioavailable and effective.




In this phase, *in vitro* cross species evaluation and metabolite identification should be performed.

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# Selection of non rodent species

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Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Review

## Comparison of minipig, dog, monkey and human drug metabolism and disposition

Lars Dalgaard *

*LD ADME Consult, Grynderupvej 13, DK-7870 Roslev, Denmark*

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# CYP activity ratios in monkeys, minipigs and dogs relative to humans

| | | Turpeinen et al. 2007 | | | Sharer et al. 1995 | | |
|---|---|---|---|---|---|---|---|
| Substrate | Human CYP | Cynomolgus monkey | Göttingen minipig | Beagle dog | Cynomolgus monkey | Rhesus monkey | Beagle dog |
| Ethoxyresorufin-O-deethylase[a, b] | 1A2 | 10 | 1 | 6 | 11 | 14 | 2 |
| Coumarin 7-hydroxylase[a, b] | 2A6 | 5 | 1 | 0.2 | 2 | 1 | 0.2 |
| Chlorzoxazone 6-hydroxylase[a] | 2E1 | 1 | 0.5 | 0.5 | NA | NA | NA |
| NDMA N-demethylase[b] | 2E1 | NA | NA | NA | 1 | 1 | 1 |
| Tolbutamide 4-hydroxylase[a, b] | 2C9 | 0.5 | 0.4 | 0.0 | 0.6 | 0.5 | 0.0 |
| Omeprazole 5-hydroxylase[a] | 2C19 | 2 | 0.2 | 0.0 | NA | NA | NA |
| S-mephenytoin 4'-hydroxylase[b] | 2C19 | NA | NA | NA | 2 | 1 | 0.3 |
| Dextromethorphan O-demethylase[a] | 2D6 | 2 | 5 | 0.4 | NA | NA | NA |
| Bufuralol 1'-hydroxylase[b] | 2D6 | NA | NA | NA | 16 | 16 | 1 |
| Midazolam 1'-hydroxylase[a] | 3A4 | 1 | 1 | 1 | 4 | 3 | 3 |
| Erythromycin N-demethylase[b] | 3A4 | NA | NA | NA | 19 | 13 | 6 |
| Omeprazole sulphoxidation[a] | 3A4 | 1 | 0.2 | 0.1 | NA | NA | NA |

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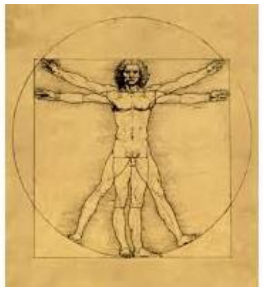
# CYP3A4

CYP3A4 is responsible for metabolism of the majority of drugs



The presence of a CYP3A4-like form in minipig liver microsomes has been demonstrated with comparable levels and activities (P. Anzenbacher *et al*. 1998).

| | | Turpeinen et al. 2007 | | | Sharer et al. 1995 | | |
|---|---|---|---|---|---|---|---|
| Substrate | Human CYP | Cynomolgus monkey | Göttingen minipig | Beagle dog | Cynomolgus monkey | Rhesus monkey | Beagle dog |
| Midazolam 1'-hydroxylase[a] | 3A4 | 1 | 1 | 1 | 4 | 3 | 3 |
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BCPT Basic & Clinical Pharmacology & Toxicology

*Basic & Clinical Pharmacology & Toxicology*, 2014, **114**, 387–394 Doi: 10.1111/bcpt.12173

# Ontogeny of CYP3A and P-Glycoprotein in the Liver and the Small Intestine of the Göttingen Minipig: An Immunohistochemical Evaluation

**Els Van Peer, Evy Verbueken, Moayad Saad, Christophe Casteleyn, Chris Van Ginneken and Steven Van Cruchten**

Applied Veterinary Morphology, Department of Veterinary Sciences, University of Antwerp, Wilrijk, Belgium

(Received 12 September 2013; Accepted 29 October 2013)

Ontogeny of CYP3A enzymes in minipig liver and intestine was found to be comparable to human supporting the use of the minipig in juvenile studies.

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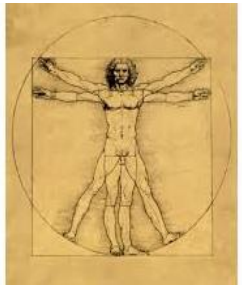
# Aldehyde oxidase (AO)

AO is an enzyme present in cytosol with emerging importance in drug discovery



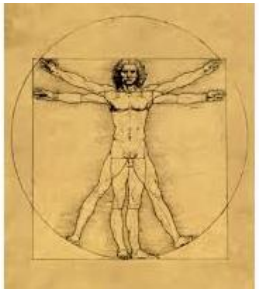
Humans synthetise a single functional aldehyde oxidase (AOX1)

**Dogs do not produce** hepatic aldehyde oxidase

The lack of this enzyme determines for example a different metabolism of vitamin A in dogs

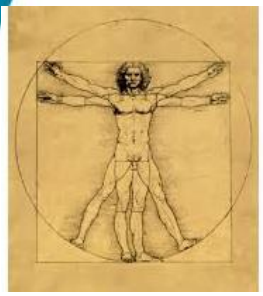
An active enzyme was shown and isolated from pigs and new genomic data demonstrate the presence of an active enzyme orthologous to the human AOX1

# Conjugation reactions



- ✓ Minipig is able to catalyze conjugation with glucuronic acid and there is evidence that glucuronosyltransferase reactions are elevated compared to human
- ✓ Minipigs exhibit a decreased level of sulphate conjugation

# N-acetyl transferase (NAT)



NAT1 and NAT2 with genetic polymorphism

- **Slow acetylators** are more prone to develop bladder cancer induced by aromatic amines
- **Fast acetylators** might be more at risk to develop colon cancer

Cytosolic NAT enzymes are not present in dogs

Pigs exhibit NAT cytosolic liver enzymes and are slow acetylators compared to humans

# Non-P450-mediated phase I metabolism

Regarding **non-P450** mediated phase I metabolism, **ester** and **amide hydrolysis** are very important reactions to be considered.

Amides are generally used to link different parts of the molecule and are relatively resistant to hydrolysis in humans.

Significant differences in hydrolysis of these functional groups can have a profound effect on:

- exposure and duration of action
- abundance of respective metabolites
- impact on drug safety

# Non-P450-mediated phase I metabolism

Hydrolysis of amides may yield aromatic amines that could be further metabolized and potentially lead to toxicity and genotoxicity

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Xenobiotica

Xenobiotica, 2016; 46(6): 483–494
 DOI: 10.3109/00498254.2015.1089452

Taylor & Francis
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RESEARCH ARTICLE

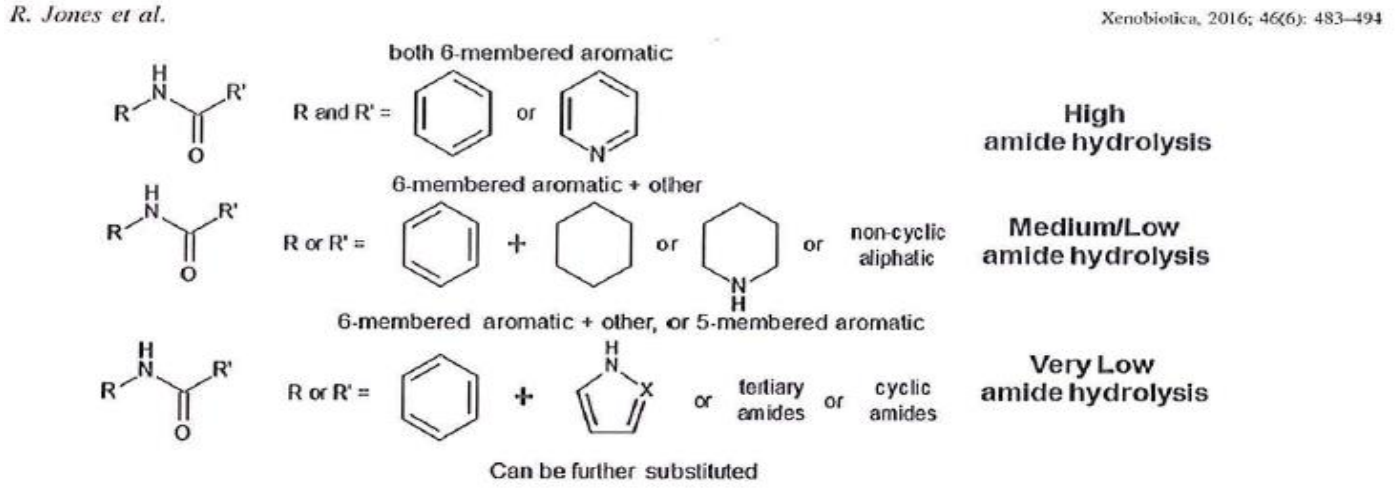
## Shedding light on minipig drug metabolism – elevated amide hydrolysis *in vitro*

Russell Jones[1], Michaela Marschmann[1], Michael Keller[2], Na Hong Qiu[1], Stephen Fowler[1], Thomas Singer[1], Franz Schuler[1], Christoph Funk[1], and Simone Schadt[1]

[1]*Pharmaceutical Sciences, Roche Pharma Research and Early Development, Roche Innovation Center Basel, F. Hoffmann-La Roche Ltd, Basel, Switzerland and* [2]*Institut fuer Pharmazeutische Wissenschaften, Albert-Ludwigs-Universitaet Freiburg, Freiburg, Germany*

# Amide hydrolysis

- Potential elevated amide hydrolysis in minipig
- SAR affecting this pathway has been established




- The activity has been localized in microsomes

This information facilitates the possible early identification and assessment of any minipig specific amide hydrolysis activity, performing a preliminary SAR analysis and microsomal clearance assays

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Pharm Res (2016) 33:2565–2579
DOI 10.1007/s11095-016-1982-5

RESEARCH PAPER

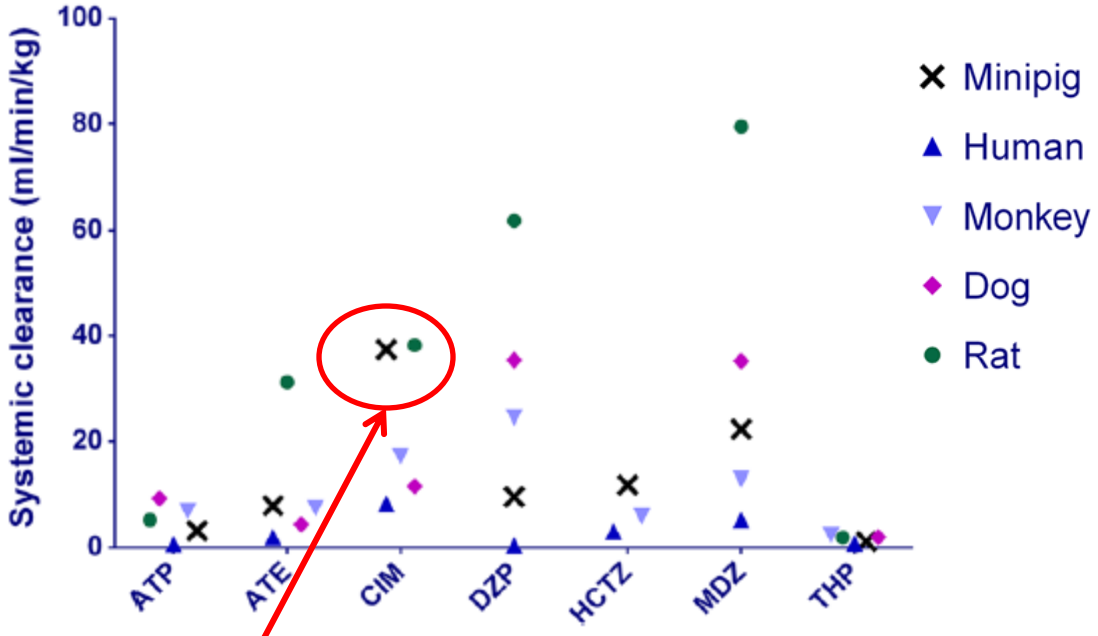
# Characterization of Pharmacokinetics in the Göttingen Minipig with Reference Human Drugs: An *In Vitro* and *In Vivo* Approach

Floriane Lignet[1] • Eva Sherbetjian[1] • Nicole Kratochwil[1] • Russell Jones[1] • Claudia Suenderhauf[2] • Michael B. Otteneder[1] • Thomas Singer[1] • Neil Parrott[1]

Seven reference compounds selected based on their absorption, metabolism and elimination routes in humans

| Antipyrine | Atenolol | Cimetidine | Diazepam | Hydrochlorothiazide | Midazolam | Theophylline |
|---|---|---|---|---|---|---|
| Several CYPs | Renal elimination | Renal elimination | CYP3A4, CYP1A2 | Renal elimination | CYP3As | CYP1A2 |

# Pharmacokinetic results




For systemic plasma clearance, values in minipig are generally lower than in rat and dog and overall more comparable to clearance observed in monkey.

In human, Cimetidine is metabolized to N'-glucuronide, sulfoxide and hydroxymethyl metabolites. The high clearance in minipig may be an indication of a high activity of these enzymes in this species.

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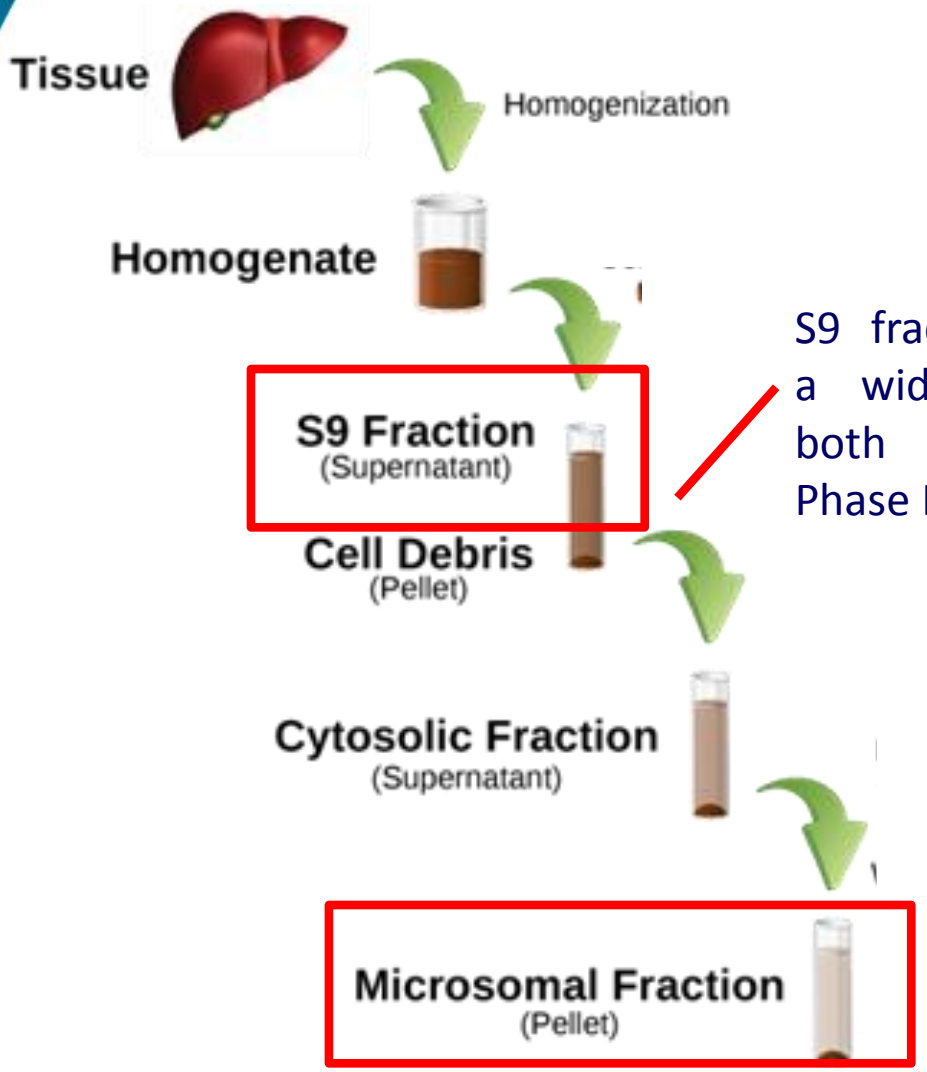
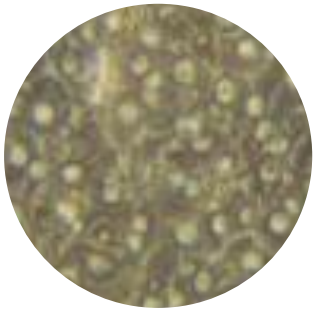
# Analytical Chemistry Staff

# Test system




S9 fraction contains a wide variety of both Phase I and Phase II enzymes

Primary hepatocytes fresh or cryopreserved



Microsomal fraction contains membrane bound enzymes: cytochrome p450 superfamily and uridine glucuronosyl transferase enzymes

# *In vitro* Metabolism - Sample Preparation

**COFACTOR MIX + TEST ITEM solution**

1°

2°

**Hepatic metabolic fraction**

T1, T2, T3, T4 and T5 samples

**INCUBATION**

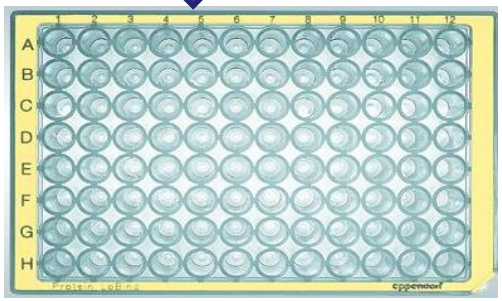
3°

4°

Time-point schedule:
T0 = 0 min
T1 = 7.5 min
T2 = 15 min
T3 = 30 min
T4 = 60 min
T5 = 120 min

**"T1" to "T5" time-points**
At the appropriate time-point, the stop solution is added and an aliquot is transferred to the corresponding well of a 96-well plate

**"T0" time-point**
An aliquot is transferred to the corresponding well of a 96-well plate; the appropriate stop solution and hepatic metabolic fraction are added




**Negative control: Cofactor medium + test item solution + buffer**

# Metabolism - Sample Preparation

- Incubation at +37°C
- under mixing at 600 rpm

**Thermo top cover is used to prevent condensation on top of the vial**

# *In vitro* Metabolism

Hepatocytes represent the "gold standard" for investigating xenobiotic biotransformation and metabolic bioactivation. They retain high levels of functionality and contain the complete set of Phase I and Phase II enzymes. When used in suspension, they provide an easy-to-handle and relatively cheap *in vitro* system that can be used for up to 4 hours

- Thawing of hepatocytes
- Trypan Blue viability determination
- Dilution at 0.5 x $10^6$ viable cells/mL

1°

2°

**TEST ITEM solutions**

3°

**INCUBATION**
**37°C, 5% $CO_2$, 95% humidity**

Time-point schedule:
T0 = 0 h
T1 = 0.5 h
T2 = 1 h
T3 = 2 h
T4 = 3 h
T5 = 4 h

**"T1" to "T5" time-points**
At the appropriate time-point, the stop solution is added. Samples are prepared for analysis.

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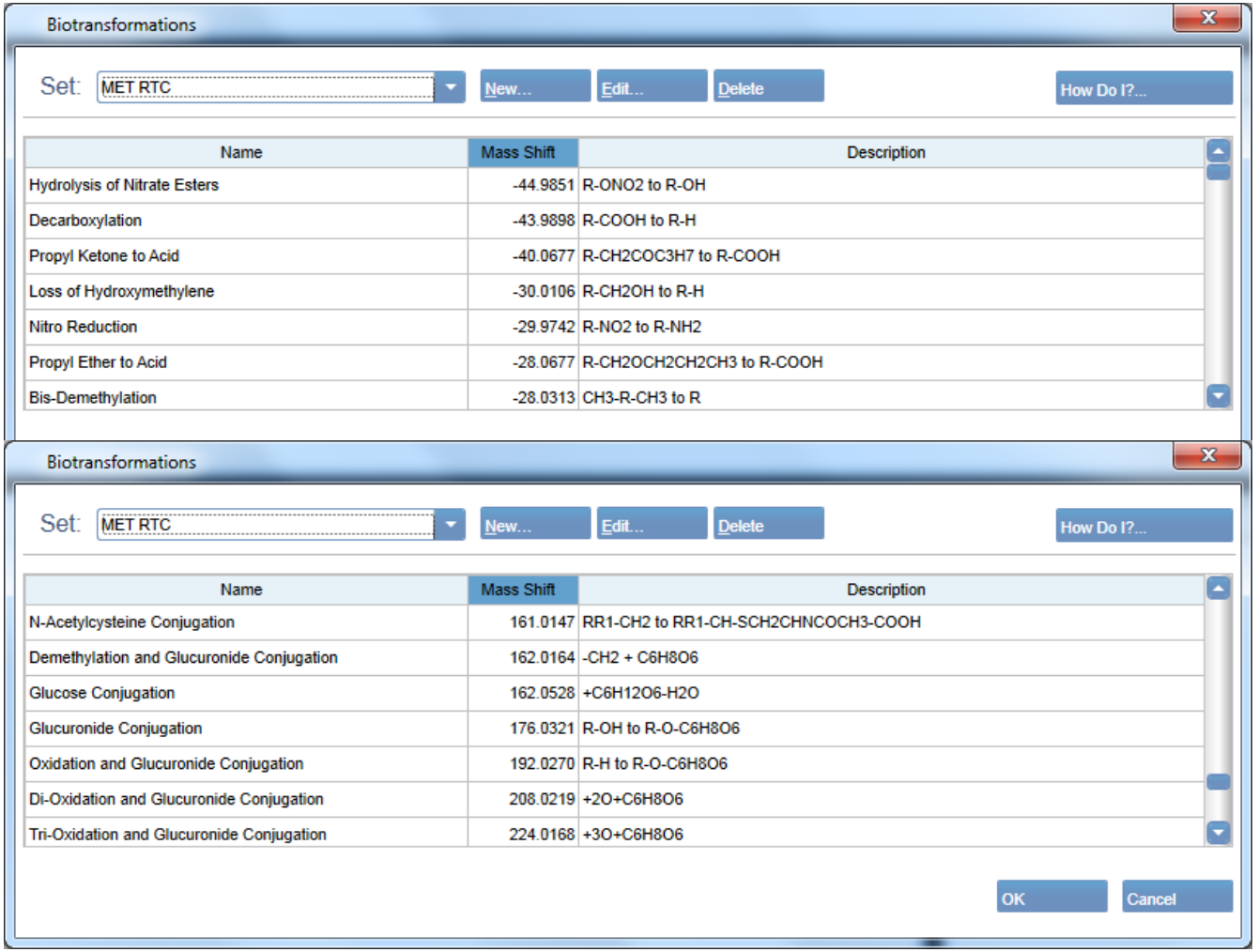
# Metabolism - Sample Analysis

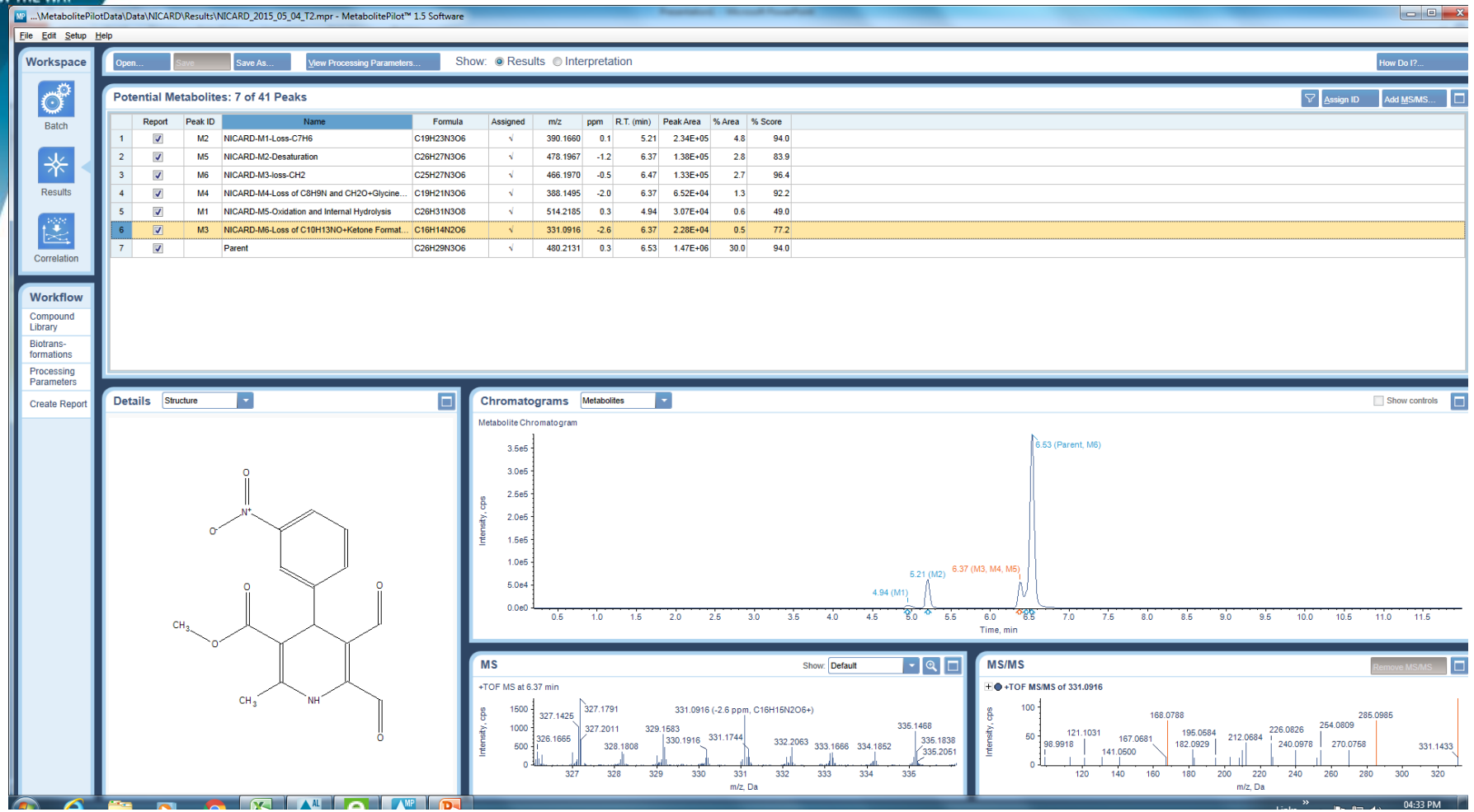
Samples are analysed using a micro liquid chromatographer coupled with a high resolution mass spectrometer

# Biotransformation set




A set of biotransformations should be selected and compared with chromatographic peaks and mass spectra

# Biotransformation results




For each metabolite the software shows the corresponding chemical structure, the chromatogram, the mass and the mass/mass spectra, that is the molecule finger printing

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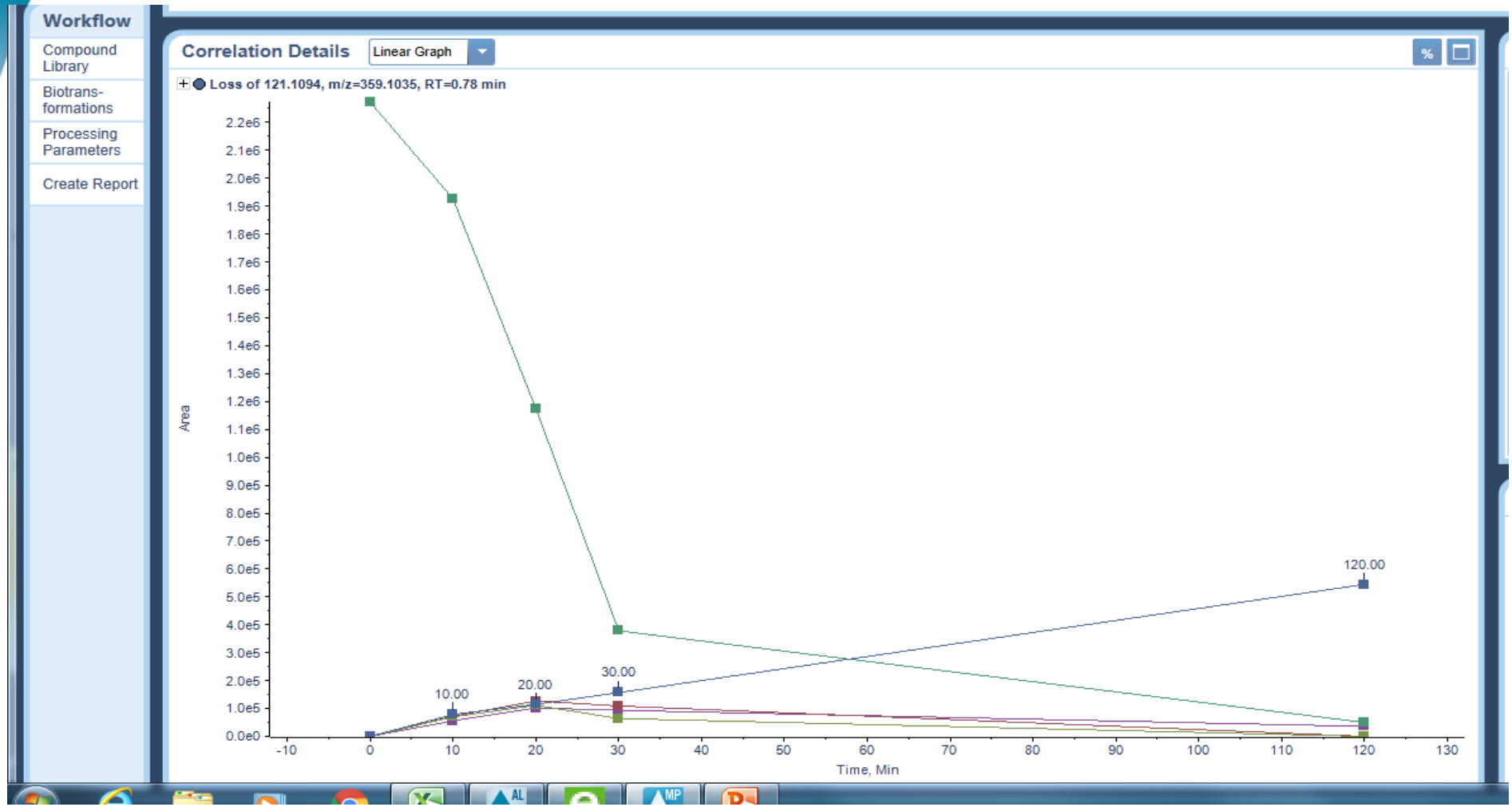
# Biotransformation results



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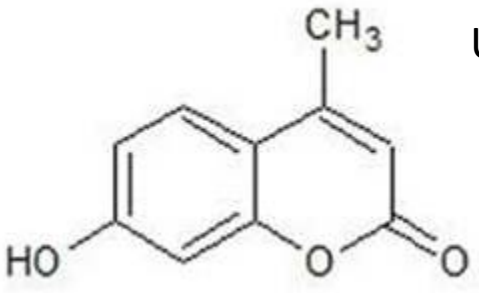
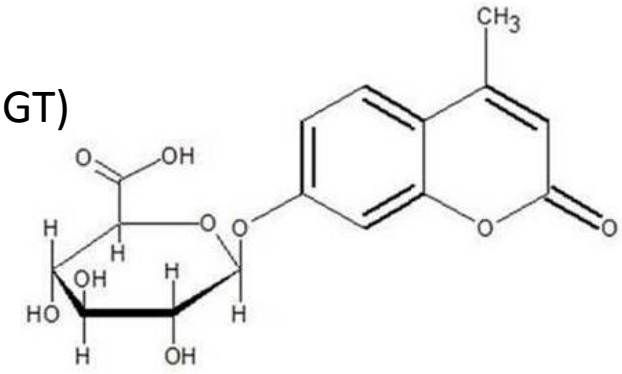
# 4-MU and its metabolites

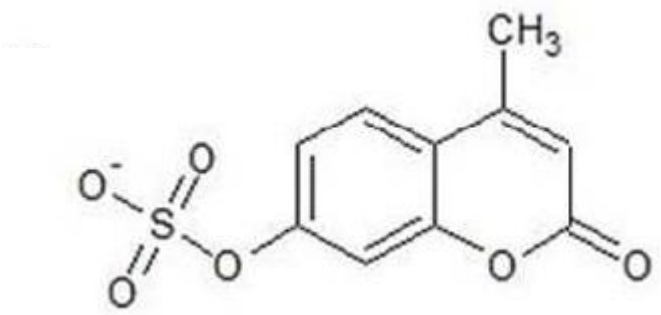
4-methylumbelliferone




UDP-glucuronosyltransferase (UGT)

4-methylumbelliferyl glucoronide




4-methylumbelliferyl sulphate

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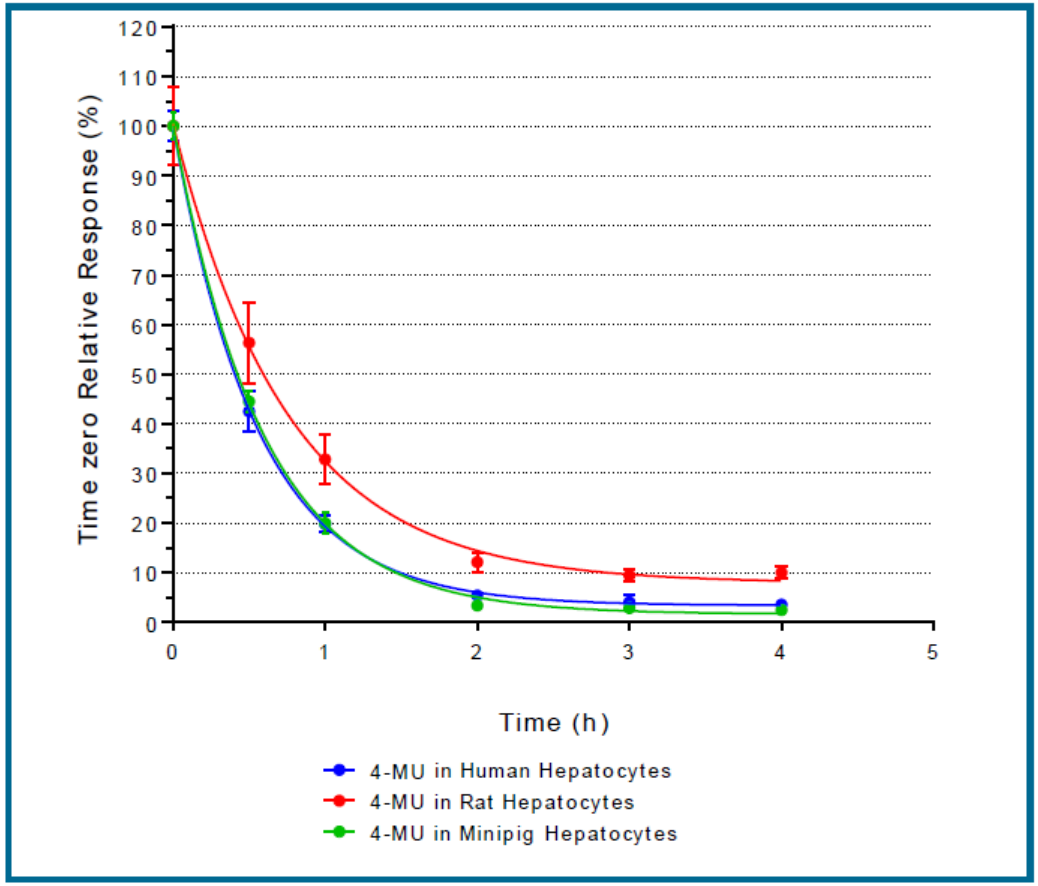
# 4-MU – Hepatocyte metabolism



# 4-MU – Hepatocyte metabolism

## Kinetic parameters

| Species | Half-life (hours) | $Cl_{int}$ (mL $h^{-1}$ $10^{-6}$ cells) |
|---|---|---|
| Human | 0.39 | 3.6 ← |
| Rat | 0.52 | 2.7 |
| Minipig | 0.41 | 3.4 ← |

In the early discovery phase, the *in vitro* intrinsic clearance is very helpful in:

- rank-ordering drug candidates based on their metabolic stabilities
- assessing species and gender differences in metabolic clearance
- projecting the metabolic clearance of drug candidates in humans

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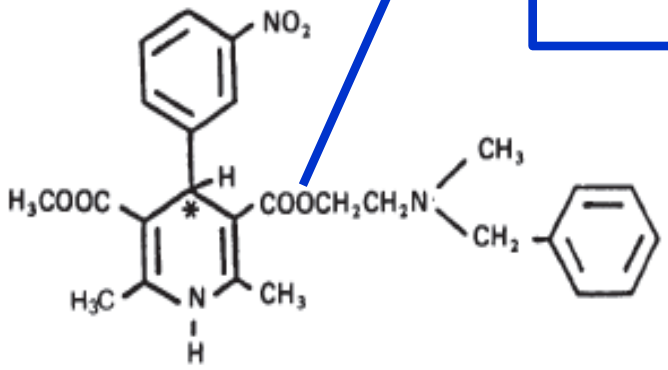
# Nicardipine and its metabolites

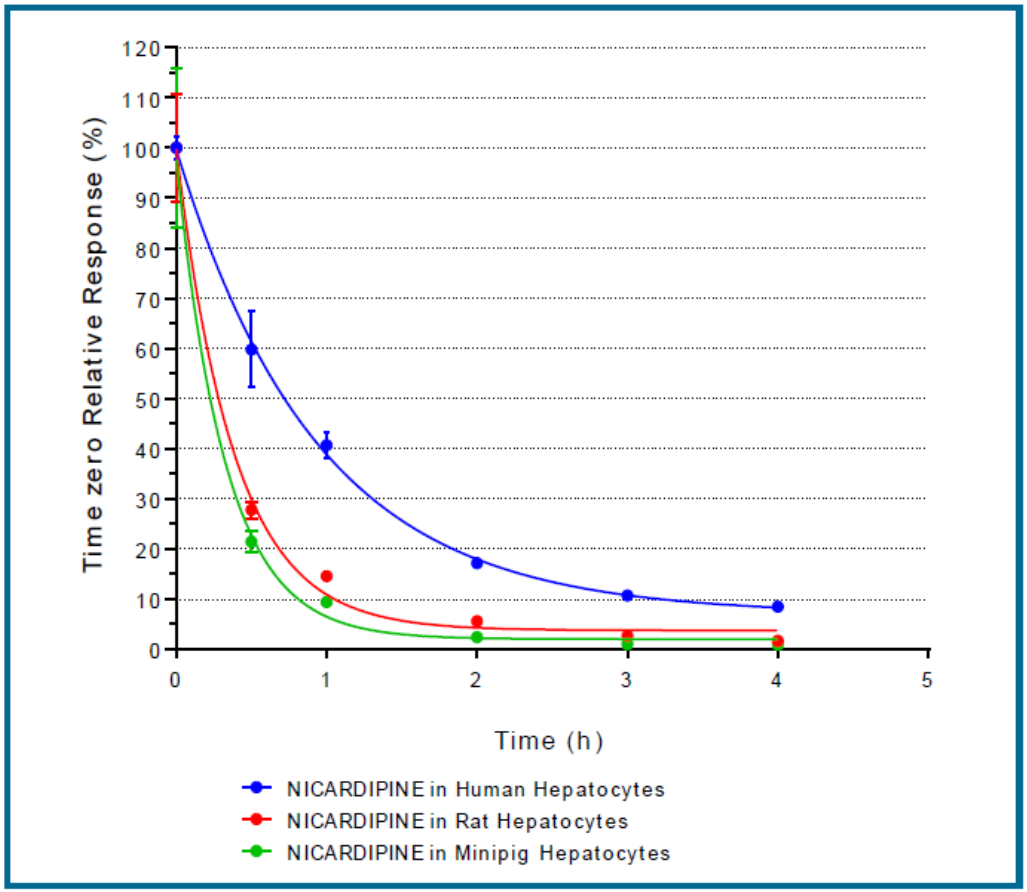
The most important reaction is the hydrolysis of the ester present in the chemical structure




Nicardipine

**CYP2C8**, **CYP2D6** and **CYP3A4** were identified as major CYP forms for the metabolism of nicardipine in human liver microsomes.

# NICARDIPINE – Hepatocyte metabolism




| Species | Half-life (hours) | $Cl_{int}$ (mL $h^{-1}$ $10^{-6}$ cells) |
|---|---|---|
| Human | 0.65 | 2.1 |
| Rat | 0.27 | 5.2 |
| Minipig | 0.23 | 6.1 |

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# NICARDIPINE Metabolism Results

Metabolite identification showed essentially a Phase I metabolism (oxidation).
The following bio-transformations are recognized:

- conversion of primary amine to alcohol
- oxidation
- loss of nitro group

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# CONCLUSIONS

✓Overall there are many good reasons to consider the minipig as a suitable model for non-clinical studies.

✓Determination of the *in vitro* intrinsic clearance and comparison of metabolite profiles, obtained using different species, is the key element for selection of relevant species to be used in toxicity testing

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# *Thank you for your attention!*

# *Any questions?*