Deuterated Drugs: Opportunities and Limits

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NJDMS, April 22nd 2010

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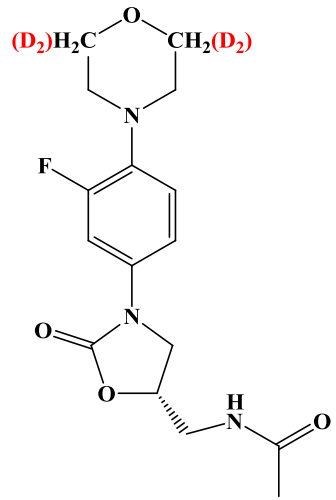
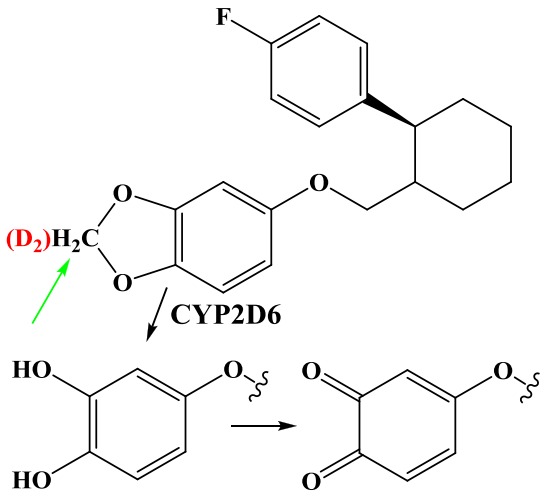
Press releases from Concert Pharmaceuticals on deuterated drugs

- Oct 27th 2008: 43% increase in preclinical (monkey) half life (4.5 to 6.3 hrs) for deuterated Linezolid
- Sept. 29th, 2009: Phase 1 start for deuterated Paroxetine;
  Expectation: attenuation of CYP2D6 inactivation
- Nov 9th, 2009: Phase 1b multiple dose escalation study for deuterated Atazanavir
  Expectation: QD dosing without Ritonavir co-administration

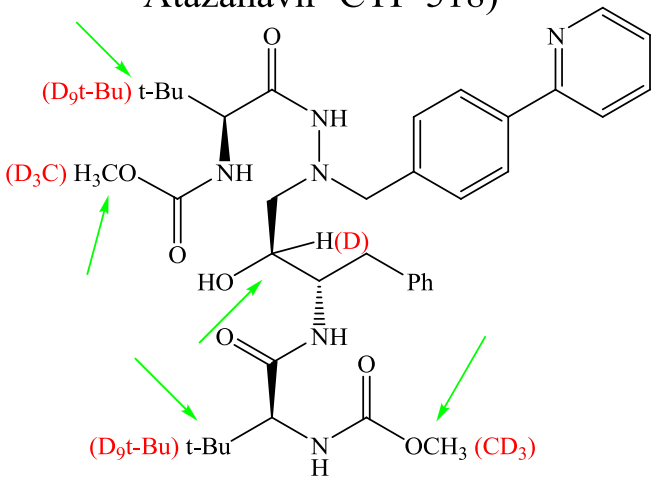
Zyvox (linezolid; C-20081)



Paroxetine (CTP307)



Atazanavir CTP-518)



How does deuterium substitution work?

- The Kinetic Deuterium Isotope Effect (KDIE)
  - C-D bonds are stronger than C-H bonds, consequently slower kinetic reaction rates, when bond breakage is rate limiting
  - If a transition state involves a symmetrical breaking of a C-H bond, substitution of hydrogen by deuterium can slow down the reaction rate by factors of 6 – 9

    KDIE = k_H/k_D ~ 6 - 9
  - The "intrinsic clearance" KDIE reflects the extent to which hepatic metabolic clearance can be altered by deuteration[1].

$$KDIE = ({}^{D}Cl_{int} / {}^{H}Cl_{int} \text{ or } {}^{D}V/Km/{}^{H}V/Km)$$

1. Cleland W., Methods in Enzymology, Vol. 249, 341-373.

Additivity of Clearance : impact on KDIE

$$CL_{systemic} = CL_h + CL_{renal} + CL_{other}$$

$$CL_{metabolic} + CL_{bile}$$

- Clearance enzymes where KDIEs can apply
  - Cytochromes P450 1 – 9
  - Aldehyde Oxidase 4 – 6
  - Monoamine Oxidases 2 – 9
  - Alcohol/aldehyde Dehydrogenases 6 – 8
- Clearance enzymes where KDIEs do not apply
  - Flavin monooxygenases
  - Glucuronyl transferases
  - Sulfotransferases
  - Glutathione-S-transferases
  - *N*-acetyl trasferases

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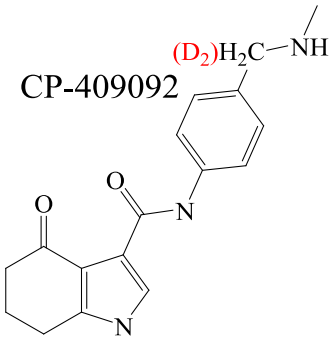
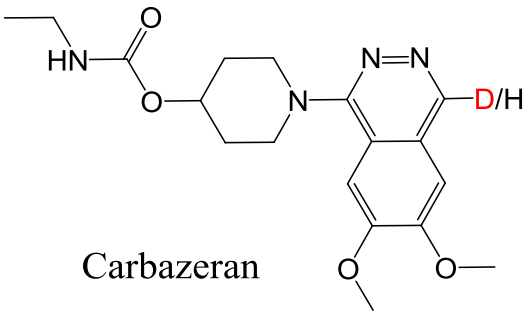
Pfizer drugs examined *in-vitro* and *in-vivo*



MAO

➢CP-409092:
- ➢Failed in FIH due to poor PK[1]
- ➢6-8 h. systemic half life in human
- ➢Poor bioavailability
- ➢MAO major contributor to metabolism



Carbazeran

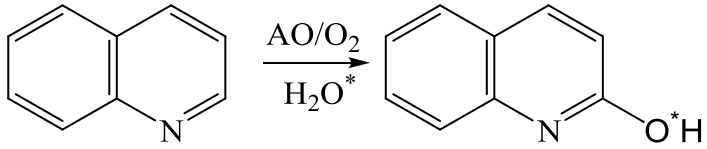
AO

➢Carbazeran:
- ➢Failed in FIH due to poor PK
- ➢Clearance greater than liver blood flow
- ➢Cleared by aldehyde[2] oxidase in guinea pig , human and rat

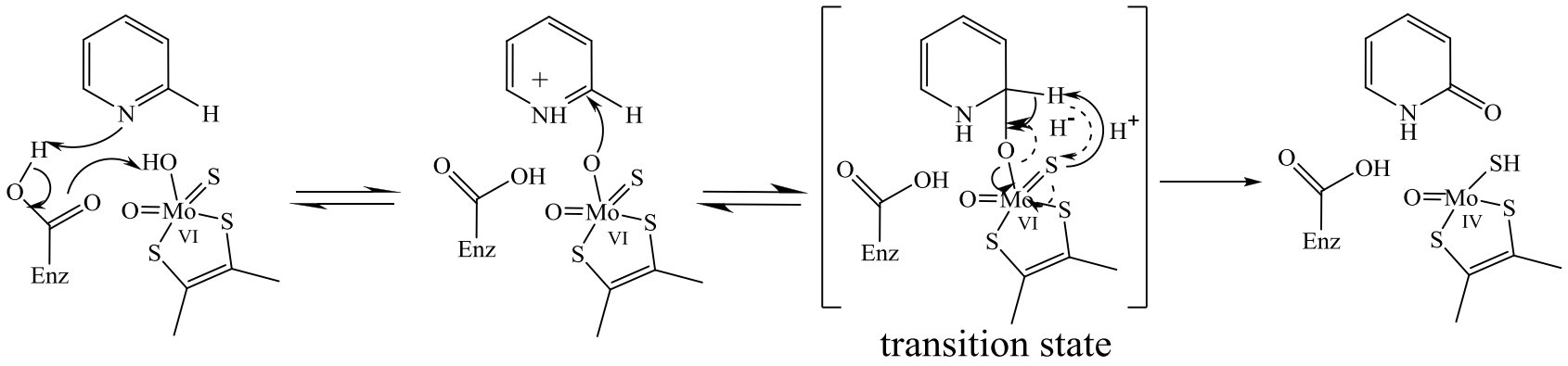
1. Sawant *et al*, 2010, *Xenobiotica*
2. Beedham C, Critchley DJ, and Rance DJ , *Xenobiotica*, 1994, Vol. 24, No. 1, Pages 37-47 .

Aldehyde oxidase

- Aldehyde Oxidase (AO), a cytosolic molybdopterin class oxidase, hydroxylates nitrogen heterocycles alpha to the nitrogen



- Wide tissue distribution
- Lacks *in vitro* to *in vivo* correlation in clearance scaling
- Experience with human PK failures due to high clearance by AO
- Proposed reaction mechanism[1] for human AO involves a rate-limiting hydride/proton abstraction in the transition state
- Potential to alter PK by use of KDIE

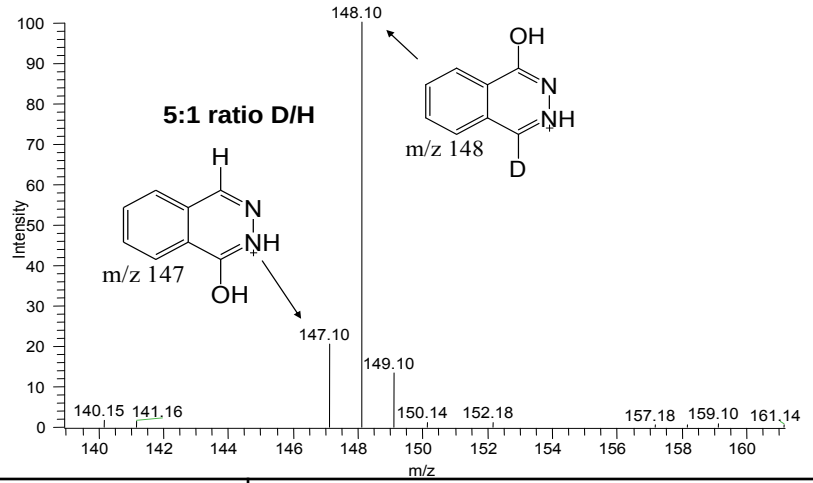


1. Alfaro, A.F. and Jones J. P. , J. Org. Chem. 2008, 73, 9469-72

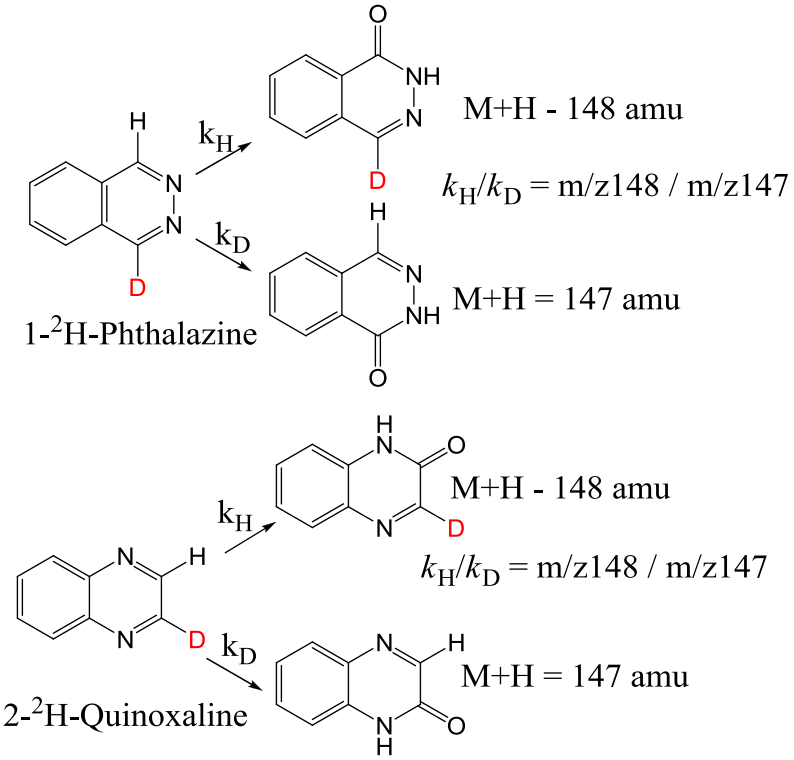
Kinetic deuterium isotope effects on aldehyde oxidase across species[1]

Intra molecular intrinsic isotope effect on AO mediated hydroxylation of mono-deuterated phthalazine and quinoxaline

Spectra of hydroxylated metabolite of pthalazine after 30 minute incubation in guinea pig/ human cytosol



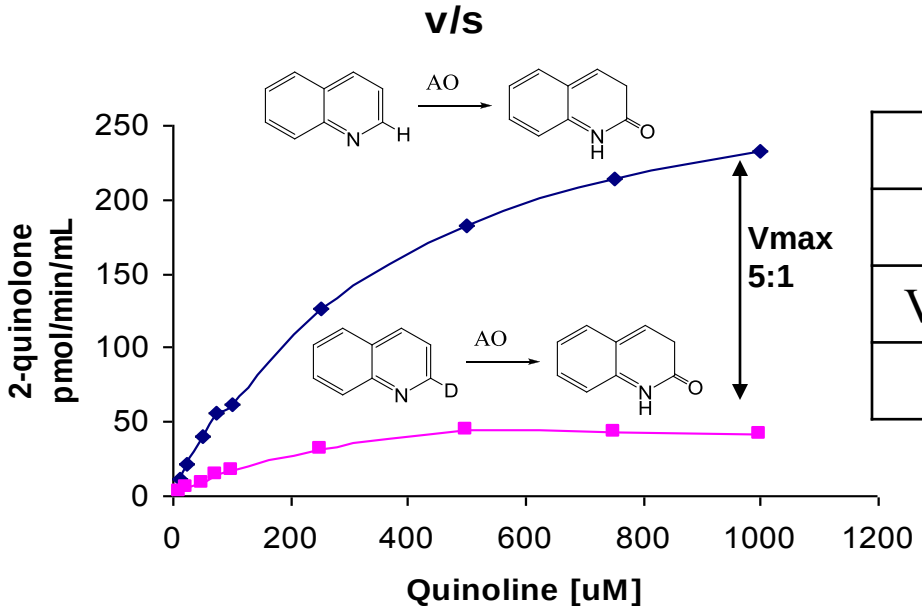
Substrate	^{H}k / ^{D}k in cytosol		
	Guinea Pig	Rat	Human
2-^{2}H-Quinoxaline	4.7	5.1	5
1-^{2}H-Phthalazine	4.9	5.0	5.1



- The rate-limiting step in AO catalyzed reactions across species is similar…hydride transfer.

Kinetic deuterium isotope effects on intrinsic clearance of aldehyde oxidase

Inter-molecular isotope effect on AO mediated hydroxylation of mono-deuterated quinoline



	Quinoline	2-^{2}H-Quinoline
K_m (mM)	212	193
V_{max} (pmol/min)	246	47
V_{max} / K_m	1.2	0.2

Conclusions:

- The rate-limiting step in AO catalyzed reactions across species is proton abstraction
- The KDIE for AO is fully expressed on the "intrinsic clearance" aka velocity of the RLS in catalysis.
- Altered pharmacokinetics should result if systemic clearance is metabolism by AO

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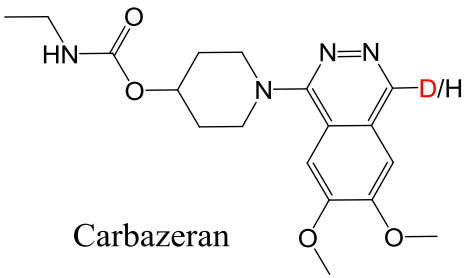
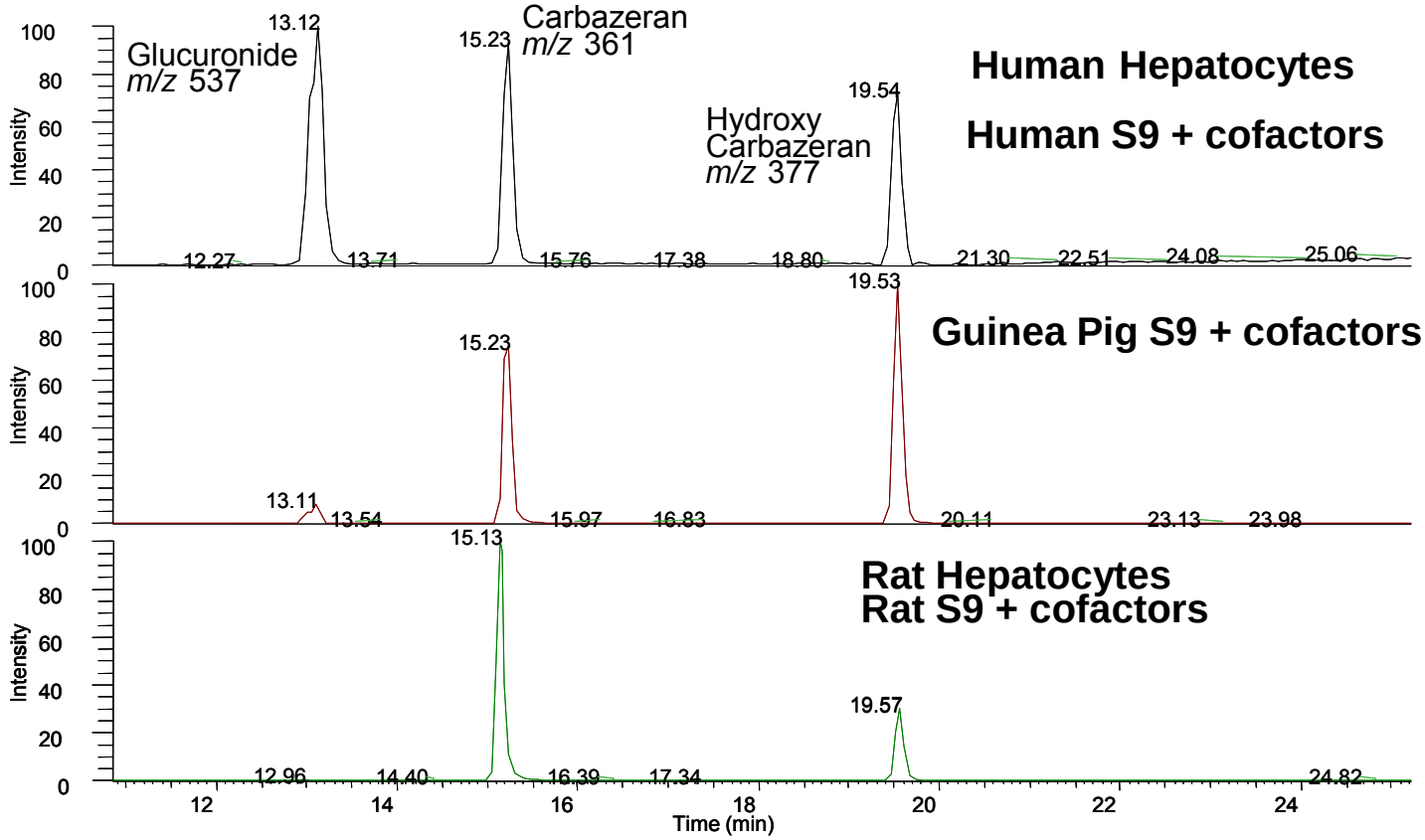
IN VITRO: Intrinsic clearance KDIE for Carbazeran

Substrate	Human		Rat		Guinea Pig	
	Cytosol	Hepatocytes	Cytosol	Hepatocytes	Cytosol	S-9
Carbazeran	4.8	1.5	5.0	4.6	6.0	5.0



Carbazeran

Metabolite profile for carbazeran



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Prediction of pharmacokinetic outcomes of deutero-carbazeran

Substrate	Human		Rat		Guinea Pig	
	Cytosol	Hepatocytes	Cytosol	Hepatocytes	Cytosol	S-9
Carbazeran	4.8	1.5	5.0	4.6	6.0	5.0

- In guinea pig and rat:
  - Increase in Cmax and AUC for oral dosing
  - May or may not change elimination half-life

- In human:
  - No significant PK enhancements
    - Low intrinsic clearance isotope effect in hepatocytes and multiple metabolic pathways predict no gain from deuteration of AO metabolic site.

KDIE on PK parameters for Carbazeran in Rats

In vitro, k_H/k_D = 4-5

IV

KDIE (D/H)		
	AUC $_{(0\text{-tlast})}$	T1/2
Rat#1	1.41	0.97
Rat#2	2.42	1.40
Rat#3	2.17	1.07
Mean	**2.0**	**1.1**
Std. Dev.	**0.5**	**0.2**

PO

	KDIE (D/H)		
	AUC $_{(0\text{-tlast})}$	T1/2	C_{max}
Rat#1	2.0	1.2	1.5
Rat#2	2.4	1.3	1.5
Rat#3	2.4	1.3	1.5
Mean	**2.3**	**1.27**	**1.5**
Std. Dev.	**0.2**	**0.1**	**0.0**

A small deuterium isotope effects on PK parameters observed in rat, consistent with a blood flow limited clearance.

KDIE on PK parameters for Carbazeran in GPs

In vitro, k_H/k_D = 5-6

IV

	KDIE (D/H)	
	AUC	**T1/2**
GP#01	8.2	0.7
GP#02	4.1	0.8
GP#03	5.4	0.9
GP#04	4.3	0.8
Mean	**5.5**	**0.8**
Std. Dev	**1.9**	**0.1**

PO

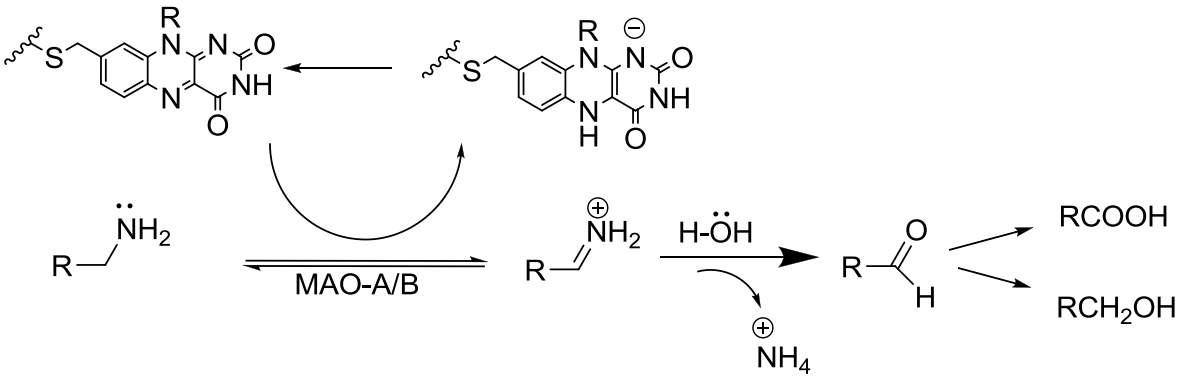
	KDIE (D/H)		
	AUC	**T1/2**	**Cmax**
GP#01	24.3	0.4	31.9
GP#02	20.2	0.6	13.4
GP#03	23.8	0.6	20.8
GP#04	19.1	0.6	23.9
Mean	**21.9**	**0.5**	**22.5**
Std. Dev	**2.6**	**0.1**	**7.7**

Conclusions for Carbazeran *in-vitro and in-vivo*

- Guinea pig shows a larger than theoretical KDIE on AUC and C_{max}, suggesting a large KDIE on the first pass extraction;
- Despite a common metabolic pathway, the guinea pig and rat differ in the outcome of KDIEs on the pharmacokinetic parameters, suggesting that species differences exist in their systemic clearance mechanisms

NO SIGNIFICANT PK ENHANCEMENT ACHIEVABLE IN CLINIC BY DEUTERATION OF CARBAZERAN AT THE AO SITE OF METABOLISM

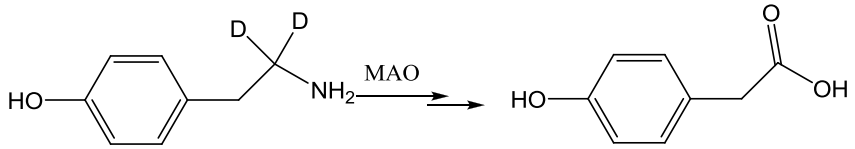
Kinetic deuterium isotope effects with monoamine oxidase



Species differences for MAOs[1]:

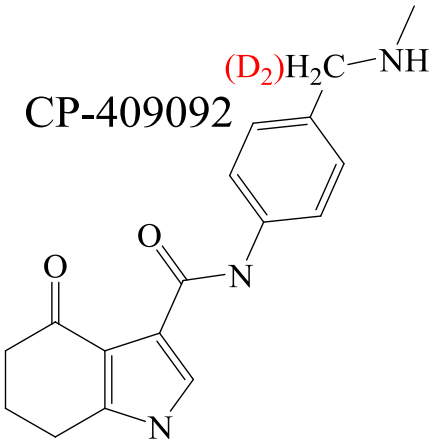
- MAO-B was the principal enzyme found in all preparations; ratio of activity of MAO-B/MAO-A ~ 3.
- Greater similarities in the MAO-A/B activities between humans and rodents than that between humans and subhuman primates.
- Very low or no expression of MAO-A in dog and monkey; but very high activity of MAO-B.

PK Enhancement by deuteration of metabolic hotspot of MAO?



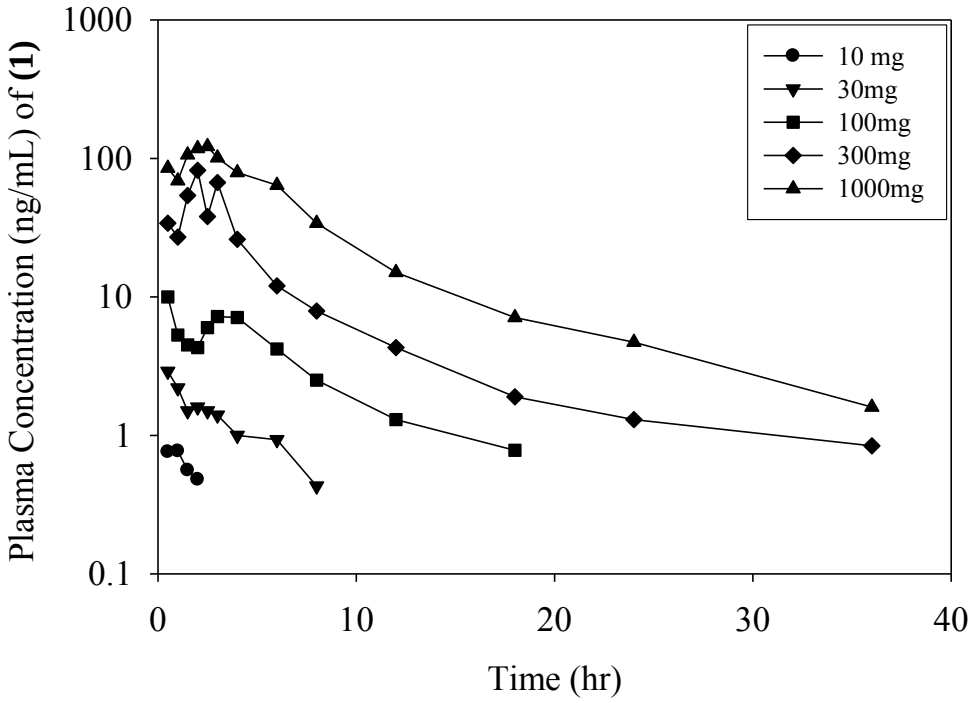
1. Castagnoli N. *et al, JPET* 1999
2. Belleau, B.; Burba, J.; Pindell, M.; Reiffenstein, J. Science (1961), 133 102-4.

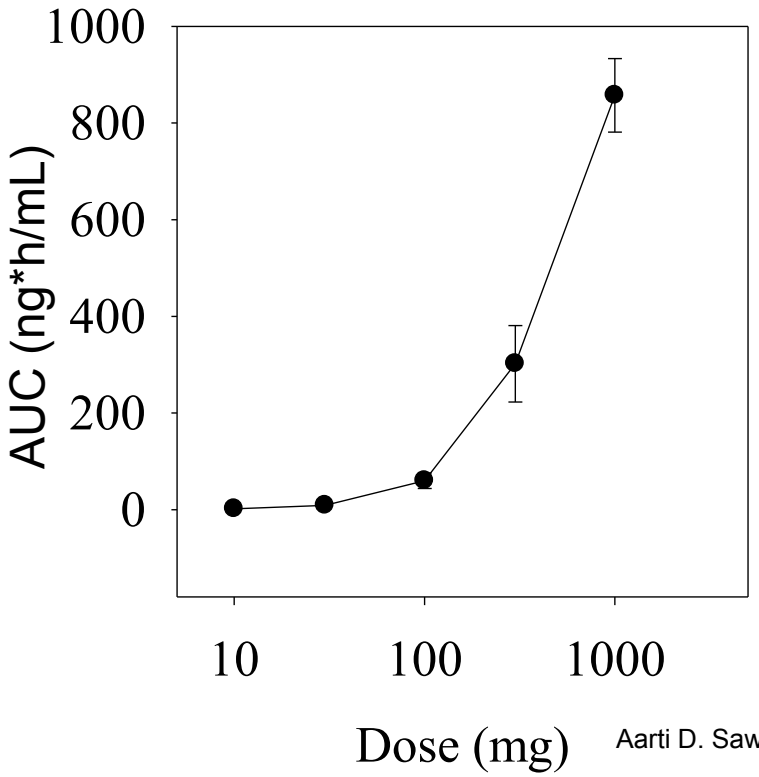
Clinical pharmacokinetics of CP-409092[1]



Known attributes:

- MAO-A substrate
- Un-desirable clinical PK
- characterized by Clp/F> 200mL/min/kg;
- Plasma elimination $t_{1/2}$: 6-8 h
- Large variability in PK

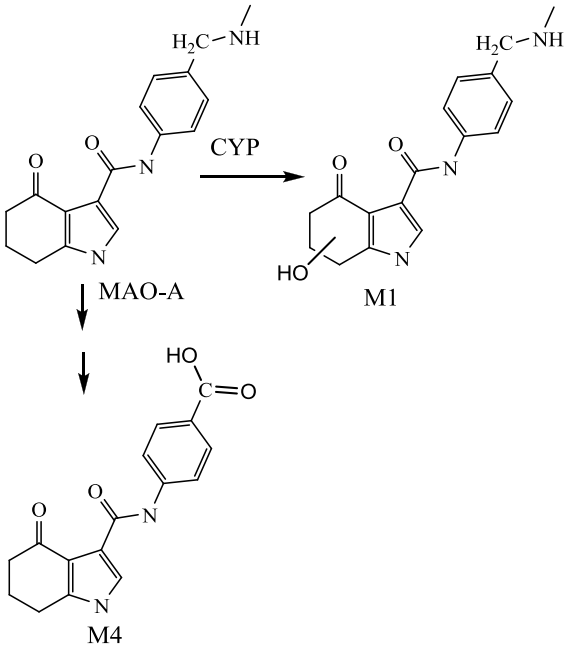




1. Sawant *et al*, 2010, *Xenobiotica*

KDIE in rat *in vitro* systems for CP-409092

System	CP-409092 Half life (min)		Isotope effect
	CP409092 proto	CP409092 deutero	k_H/k_D
*h*MAO-A membranes	36.4	138.6	3.8
Rat Hepatocytes	71	99	1.4



Radiolabel Disposition of CP-409092 in Rat[1]

Metabolites	Urine (U)	Feces (F)	U+F
M1A	0.06	ND	0.06
M1	0.74	6.47	7.21
M1B	0.04	0.62	0.66
M2	0.88	3.5	4.39
M2A	0.08	ND	0.08
M4 (acid metabolite)	0.62	3.24	3.86
M5	0.02	0.22	0.24
(unchanged drug)	1.01	72.4	73.41
Total	3.45	66.5	89.9

Solubility: > 5 mg/mL;
$Caco_2$: Papp AB : 1.2x 10^{-6}; Papp: **BA/AB: 7**

Undesireable PK:

1. Kamel *et al* 2010

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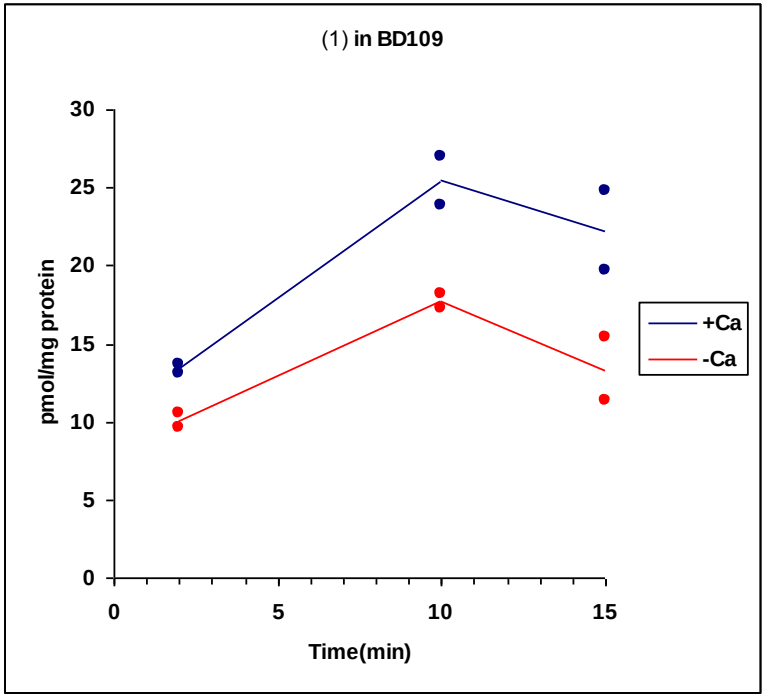
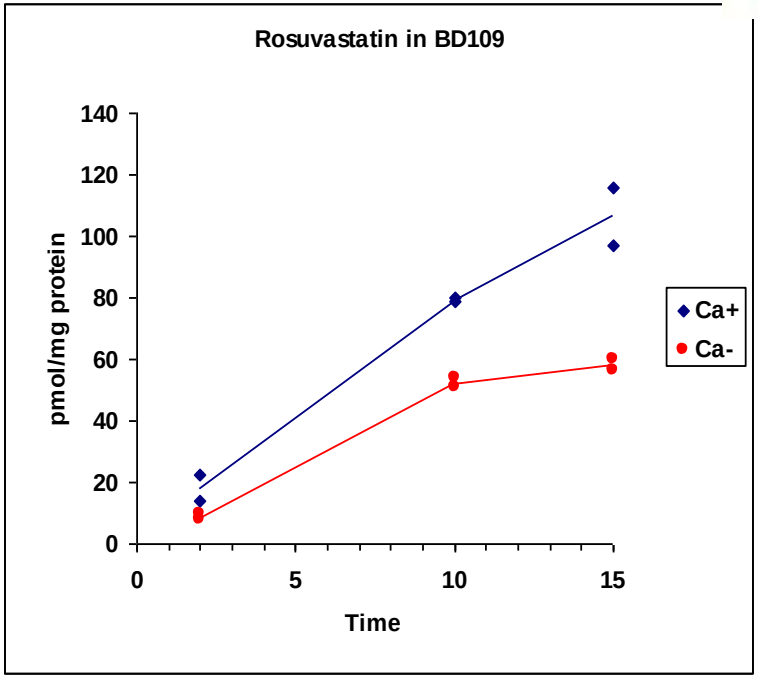
Biliary uptake in Sandwich cultured human hepatocytes





	Uptake, $_{app}$ (pmol/min/mg)	Cl_b (uL/min/mg protein)	BEI (%)
Rosuvastatin[1]	7.7	2.7	34
CP-409092[2]	1.5	0.77	30

1. Martin et al., 2003 *Clin. Ther.* **25** (2003), p. 2822.
2. Sawant *et al*, 2010, *Xenobiotica*

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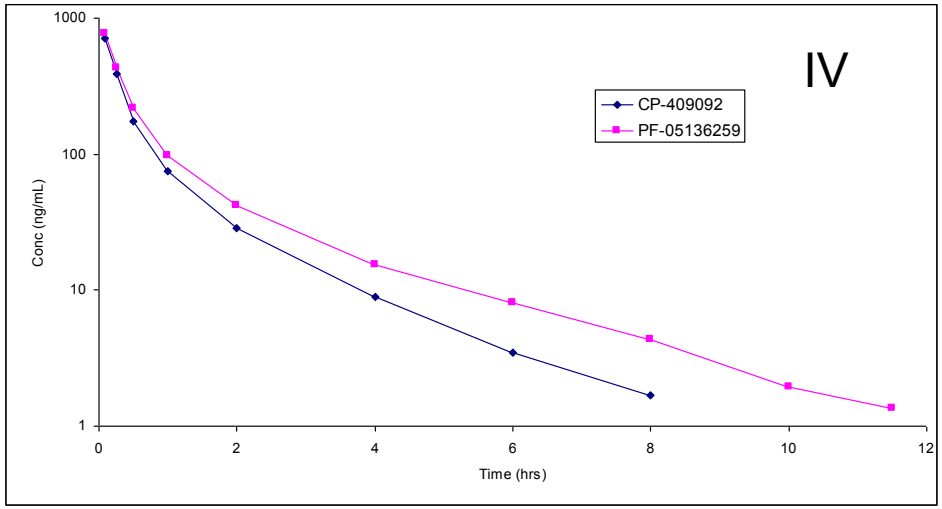
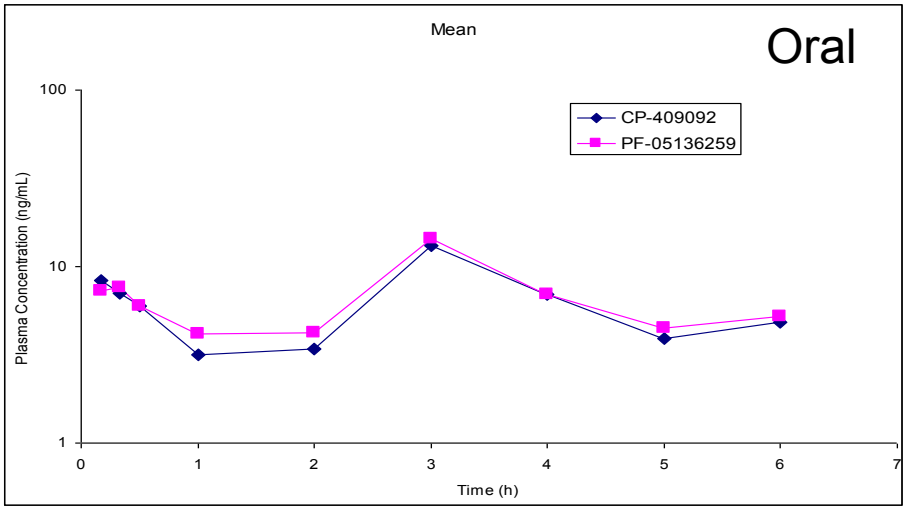
Rat IV and oral pharmacokinetic isotope effects for CP-409092





	$AUC_{(D)}/AUC_{(H)}$	$T_{(1/2)(D)}/T_{(1/2)(H)}$	$C_{(max)(D)}/C_{(max)(H)}$
R-01	1.3	1.3	1.1
R-02	1.2	1.1	0.8
R-03	1.2	1.2	1.0

Overall, deuteration of the alpha position of the benzylic group did not result in PK enhancement in rat, in vivo.

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KDIE in human *in vitro* systems for CP-409092

System	CP-409092 Half life (min)		Isotope effect
	proto	deutero	k_H/k_D
HLM – NADPH (10)	133	577	4.3
HLM + NADPH (10)	182	693	3.8
Hepatocytes (1/10)	96	551	5.7/7.4

- Large KDIE in human in vitro systems suggest possible PK enhancement
- However, presence of other clearance routes (biliary/absorption) may not favor overall enhancement of pharmacokinetic parameters

Summary

- As shown with two substrates (CP-409092 and Carbazeran), deuteration as a strategy to enhance pharmacokinetic parameters requires:
  - Disposition of candidate across species;

    $(CL_{systemic} = CL_h + CL_{renal} + CL_{other})$
  - The identity of enzymes involved in the metabolic clearance
  - Knowledge of their reaction mechanisms
  - The extent of their contribution to the overall metabolic clearance

THANK YOU!

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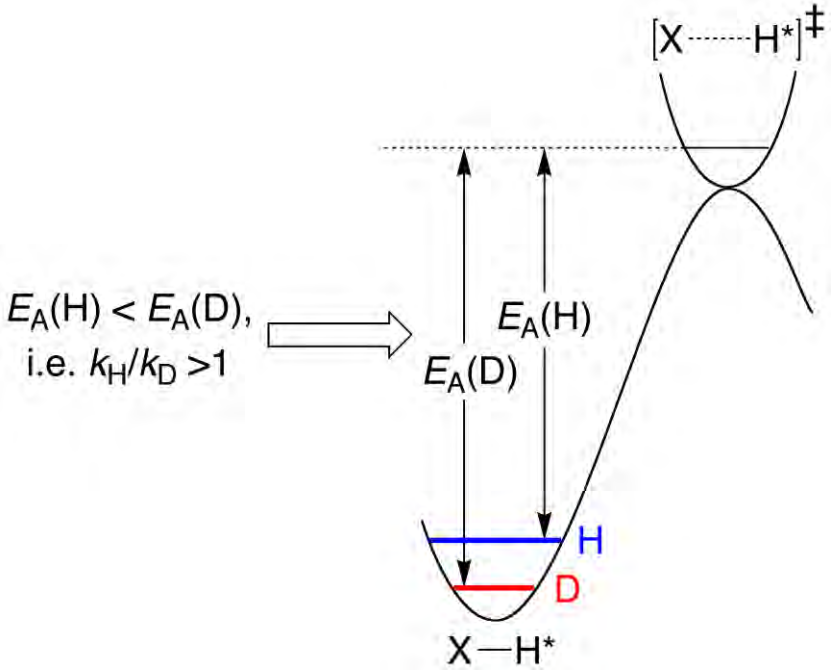
Use of Deuterated Drugs

- As an internal standard in quantitative analysis
  - Three or more non-exchangeable deuterium atoms incorporated to mass-differentiate an internal standard from the analyte in SRM or MRM transitions

- Alter a drugs pharmacokinetic or toxic properties
  - One or more deuterium atoms substituted at specific sites may slow metabolism
    - decrease a drugs metabolic clearance resulting in
      - An increase in C_{max}, AUC and systemic half-life ($T_{1/2}$)
    - decrease metabolically generated toxic metabolites.

How does deuterium substitution work?

- The Kinetic Deuterium Isotope Effect (KDIE)
  - C-D bonds are stronger than C-H bonds, consequently slower kinetic rates by factors of 6 – 9 when bond breakage is rate limiting



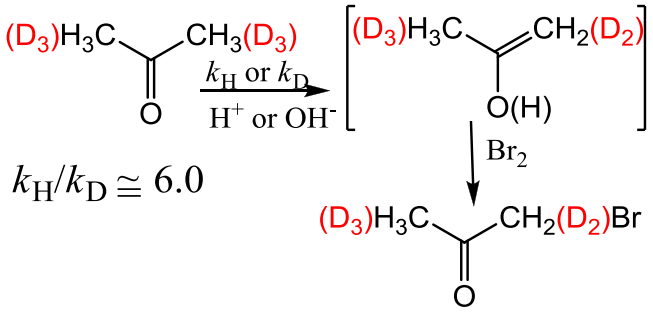
If a reaction transition state involves a symmetrical breaking of a C-H bond, substitution of hydrogen by deuterium can slow down the reaction rate by factors of 6 – 9

$$\text{KDIE} = k_H/k_D \sim 6 - 9$$

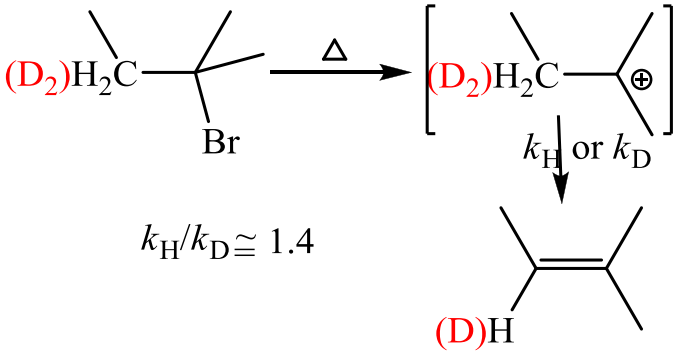
Applications of kinetic deuterium isotope eff

In Chemistry:

The KDIE identifies rate limiting steps



$k_H/k_D \cong 6.0$



$k_H/k_D \cong 1.4$

In Enzymology[1]:

$$E + [S\} \rightleftharpoons [ES_1] \overset{{}^{H}k \text{ or } {}^{D}k}{\rightleftharpoons} [ES_2] \rightleftharpoons [ES_3] \xrightarrow{k_{cat}} E + P$$

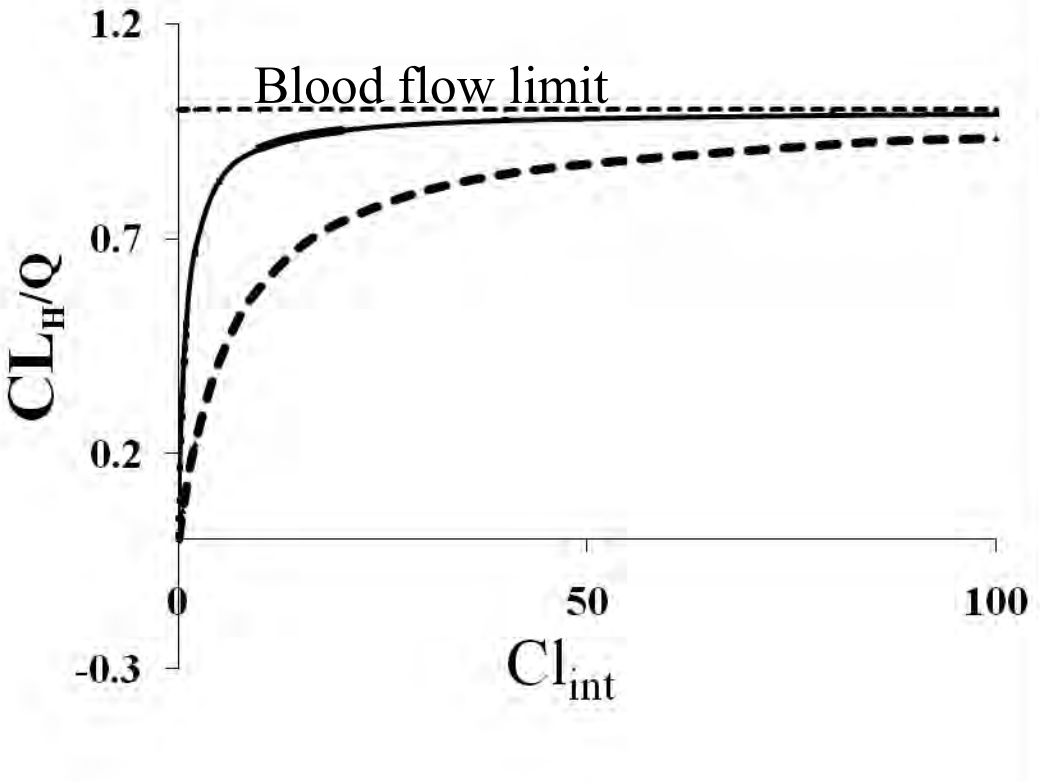
$^{H}k/^{D}k$ = the "intrinsic" KDIE or "commitment to catalysis" parameter reflects the irreversibility of the bond breakage step

$^{H}k_{cat}/^{D}k_{cat}$ reflects the extent to which bond breaking limits catalysis

1. Cleland W., Methods in Enzymology, Vol. 249, 341-373.

Theoretical relationship of an effect on hepatic (metabolic) clearance (CL_H) for a KDIE on intrinsic clearance (Cl_{int})

$$CL_H = \frac{Q_H \times Cl_{int}}{Q_H + Cl_{int}}$$



If $Cl_{int} >> Q_H$,

➢For an orally dosed drug a KDIE on the Cl_{int} will affect the extraction ratio (first pass effect) resulting in AUC and C_{max} increases but no change in systemic half life

If $Cl_{int} << Q_H$,

➢For an IV or PO dosed drug a KDIE on the Cl_{int} will increase AUC, C_{max} and systemic half life