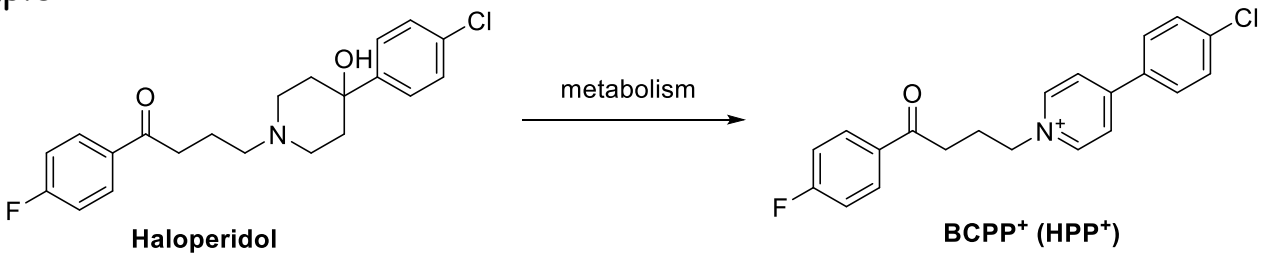
- Metabolic breakdown of drugs by living organisms, usually through specialized enzymatic systems.

Drug (liphophilic) —Enzyme→ Metabolite (polar) → Excretion

- It may lead to the formation of inactive and non-toxic compounds, hence the term detoxification.
- Some studies have shown that some metabolites are not only active, but may be toxic.

Example:



Haloperidol **BCPP$^+$ (HPP$^+$)**

Igarashi, K. *Life Sci*. **1995**, 57, 2439.
Sampson, D. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 4294.

Functional outcomes of drug metabolism:

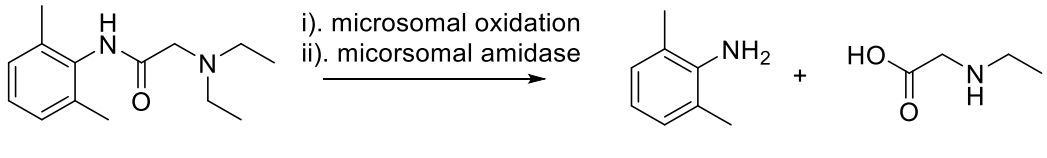
- Inactivation and accelerated elimination of drugs.
- Activation of prodrugs.
- Formation of active metabolites with similar or novel activity.
- Detoxification of toxic xenobiotics
- Toxification of non-toxic xenobiotics.

Roskoski, J. *Pharmacol. Res.* **2016**, *107*, 249.

Site of drug metabolism:

1. Organ sites

- **Liver** : major site, most drug metabolizing enzymes.
  - orally administered drugs first pass through the liver and thus are susceptible to first-pass effect. This can lead to lower bioavailability.
  - For example, Lidocaine is inactive when given orally due to the first-pass effect.



Lidocaine

- **Other sites** : intestine, kidneys, lungs, skin, placenta, brain, adrenal glands.

2. Cellular sites: cytosol, mitochondria, lysosomes, smooth endoplasmic reticulum.

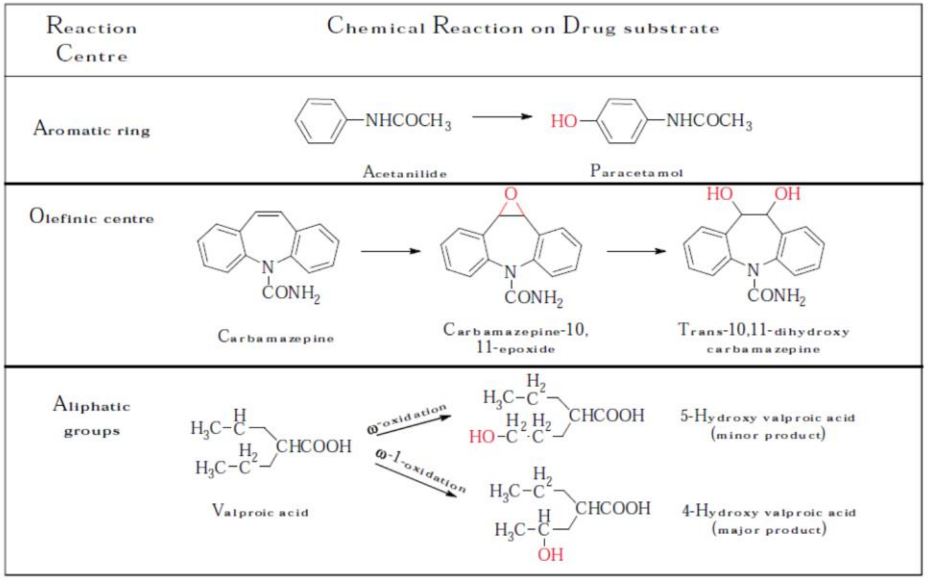
Categories of drug metabolism reactions:

1. **Phase I reactions (modification)**:

- oxidation, reduction, hydrolysis, cyclization, decyclization.
- which converts parent drug to more polar metabolite by introducing or unmasking a functional groups, e.g.: –OH, -COOH, -NH_2.
- usually involve enzymes like cytochrome P450 monooxygenase, flavin-containing monooxygenase, alcohol dehydrogenase, aldehyde dehydrogenase, monoamine oxidase, peroxidases.

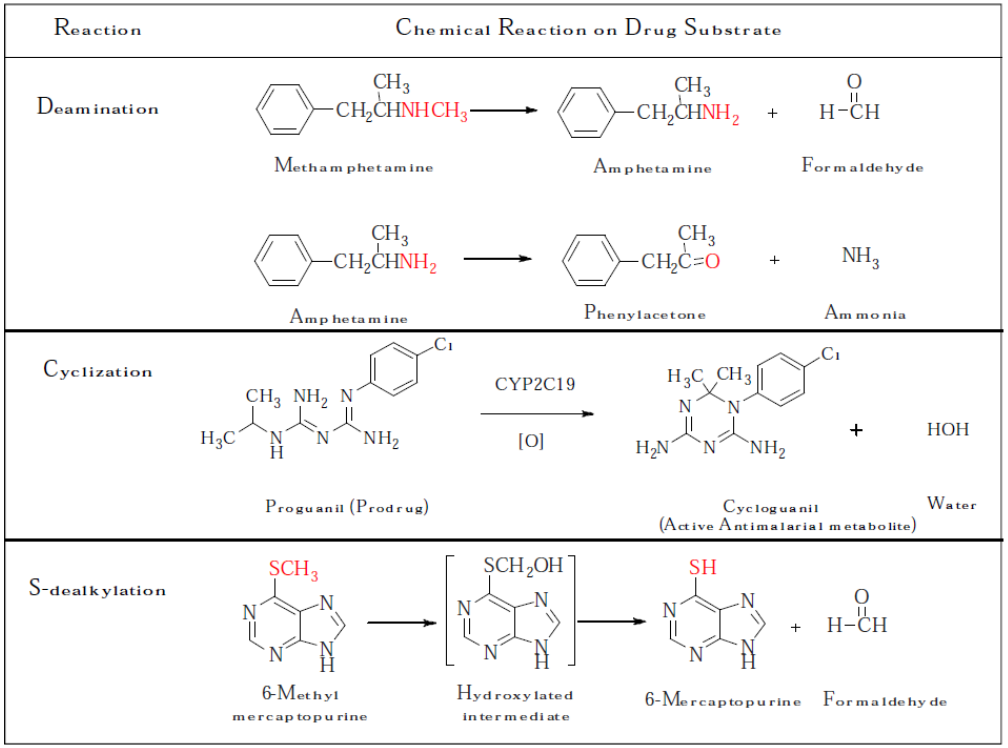
(a). **Oxidation:** may occur at several centres in drugs.

Oxidation at carbon centre: includes oxidation at aromatic ring, olefinic centre, and aliphatic groups



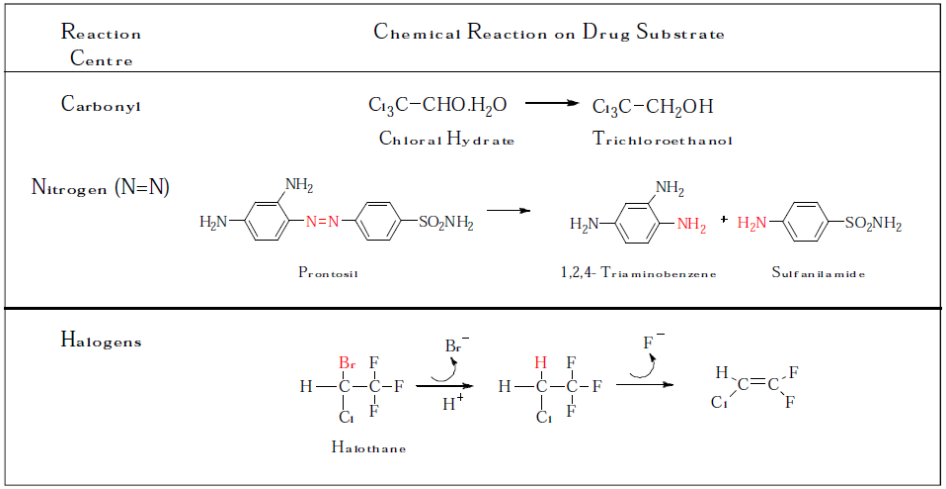
Oxidation at carbon-heteroatom systems:

- involves reaction on *C*–N, *C*–S and *C*–*O* systems.
- The oxidation reactions on *C*–N systems comprise of N-dealkylation, oxidative deamination, formation of N-oxide, or N-hydroxylation.
- The reactions in *C*–S systems may involve S-dealkylation, desulfuration and S-oxidation.
- *O*-dealkylation, oxidative dehalogenation and oxidative aromatization are other important oxidation reactions at carbon centre.



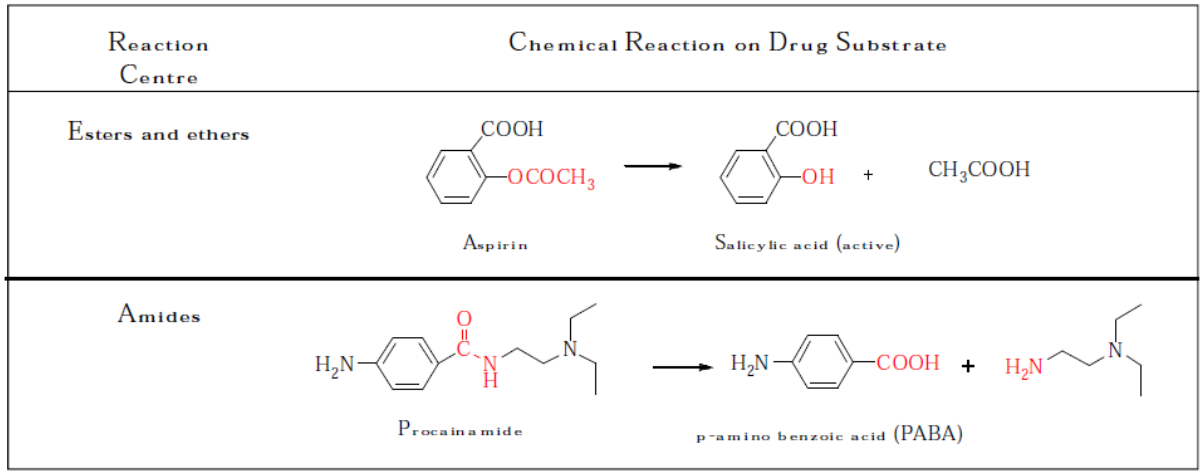
(b). **Reduction:**

- can take place in aliphatic and aromatic aldehydes and ketones.
- Drugs such as methadone (analgesic), chloral hydrate (sedative and hypnotic) and naltrexone (management of alcohol dependence) undergo this metabolic process.
- The N-containing compounds having nitro, azo or N-oxide undergo this metabolic reaction. For example: nitrazepam (hypnotic and anxiolytic) and prontosil (antibacterial antibiotic), which get reduced to the corresponding amines.
- The halogen atom present in various drugs may undergo reduction via replacement by H-atom, e.g., halothane.
- usually involve enzymes like NADPH-cytochrome P450 reductase, reduced (ferrous) cytochrome P450.



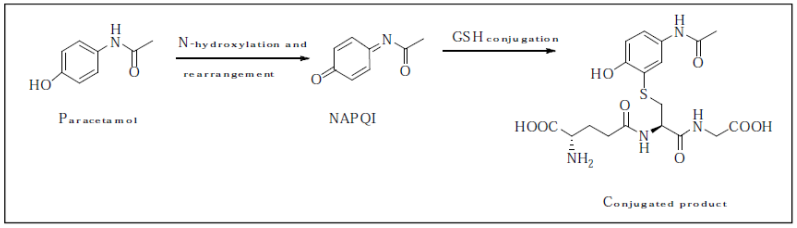
(c). **Hydrolysis:**

- esters are administered as prodrugs, which on hydrolysis are converted to active forms, e.g., aspirin (analgesic, antipyretic).
- drug molecules containing amide functionality undergo slow hydrolysis as compared to esters.
- The reaction occurs in secondary and tertiary amides, and rarely in primary amides, e.g., procainamide (antiarrhythmic).
- usually involve enzymes like esterases, amidase, and epoxide hydrolase.



2. **Phase II reactions (conjugation)**: combination type reaction. e.g. A + B = AB.

- which attaches polar and ionizable endogenous groups to achieve complete solubility, e.g.: sulfate, acetate, amino acid.
- sites on drugs where conjugation reactions occur include carboxy (-COOH), hydroxy (-OH), amino (NH_2), and thiol (-SH) groups.
- It can also lower or terminate biological activity.
- These reactions are catalyzed by a variety of transferase enzymes, such as uridine diphosphate (UDP)-glucoronsyltransferases, sulfotransferases, glutathione transferases.
- In these reactions, a suitable moiety such as glucuronic acid, glutathione, sulphate, glycine, etc., get conjugated to the metabolites of Phase I reaction.
- An example of glutathione conjugation as the Phase II metabolic reaction in the body.



Conjugated Group	Endogenous Cofactor	Enzyme	Drugs
Glucuronidation	UDP glucoronic acid	UDP-glucuronosyltransferase	Chloramphen morphine, par salicylic acid, fenoprofen, desipramine, meprobamate, cyproheptadi
Sulphation	Sulphate	Sulfotransferases	Paracetamol,
Glycine/Glutamine conjugation	Glycine/glutamine	N-acyl transferases	Cholic acid, salicylic acid, acid, phenyla
Glutathione conjugation	Glutathione	Glutathione S -transferases	Paracetamol, acid
Acetylation	Acetyl-CoA	N-acetyl transferases	Histamine, m procainamide, salicylic acid, phenelzine, h dapsone
Methylation	S-adenosyl-L-	Methyl transferase	Morphine, no

The conjugation reactions along with the enzymes involved and the examples of drugs metabolized by the same pathway.

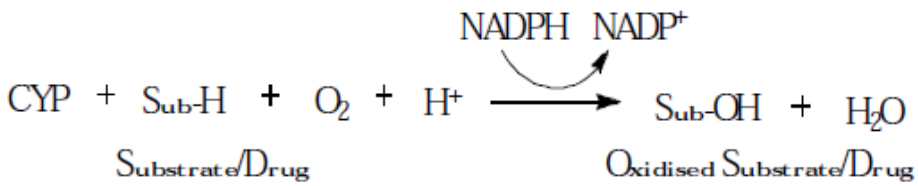
Conjugated Group	Endogenous Cofactor	Enzyme	Drugs
Glucuronidation	UDP glucoronic acid	UDP-glucuronosyltransferase	Chloramphenicol, morphine, paracetamol, salicylic acid, fenoprofen, desipramine, meprobamate, cyproheptadiene
Sulphation	Sulphate	Sulfotransferases	Paracetamol, salbutamol
Glycine/Glutamine conjugation	Glycine/glutamine	N-acyl transferases	Cholic acid, salicylic acid, nicotinic acid, phenylacetic acid
Glutathione conjugation	Glutathione	Glutathione S-transferases	Paracetamol, ethacrynic acid
Acetylation	Acetyl-CoA	N-acetyl transferases	Histamine, mescaline, procainamide, p-amino salicylic acid, isoniazid, phenelzine, hydralazine, dapsone
Methylation	S-adenosyl-L-methionine	Methyl transferase	Morphine, norephedrine nicotine, histamine, isoprenaline, propylthiouracil

3. **Phase III reactions (further modification and excretion)**:

- further metabolism of xenobiotic conjugate after phase II reactions.

Cytochrome P450 enzyme system (CYP450)

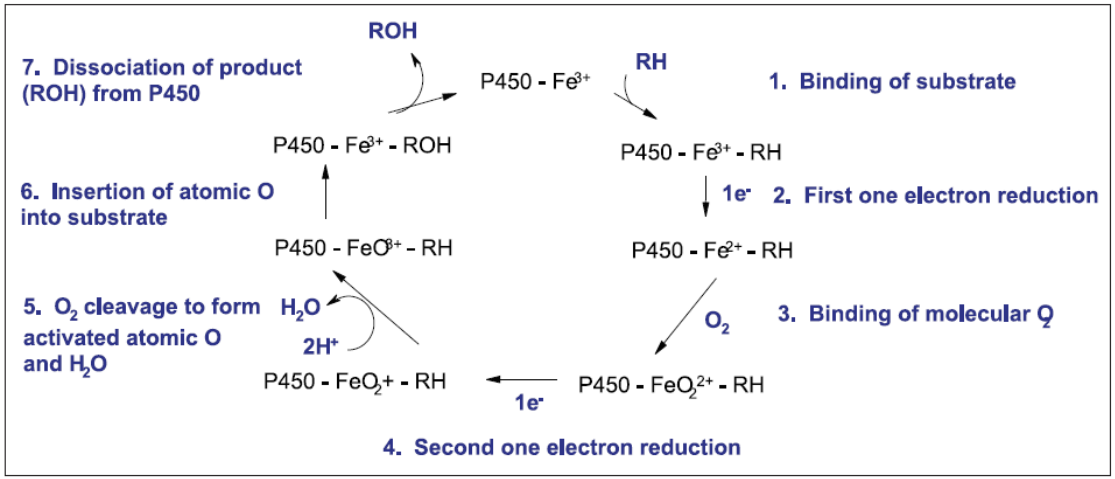
- Superfamily of enzymes
- contain a heme prosthetic group, where heme group is the iron-porphyrin unit.
- It oxidizes hydrophobic compounds to hydrophilic or more polar metabolites for subsequent excretion.
- are membrane-bound proteins, present in the smooth endoplasmic reticulum of liver and other tissues.
- CYPs catalyze the transfer of one atom of oxygen to a substrate producing an oxidised substrate along with a molecule of water.



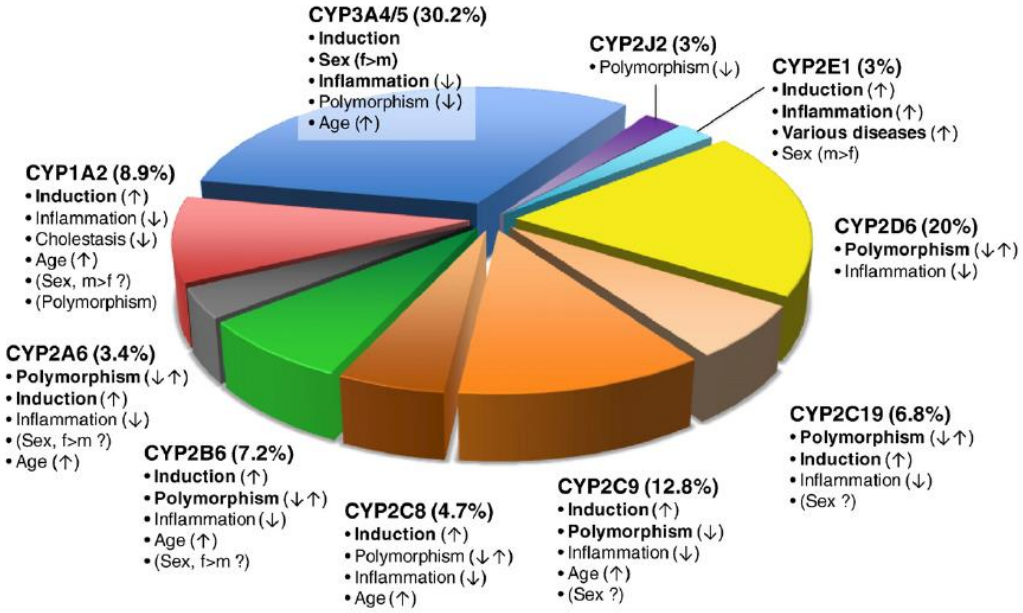
- Cytochromes P450 have been named on the basis of their cellular (cyto) location and spectrophotometric characteristics (chrome).
- Reduced heme iron combines with CO, P450 enzymes absorb light at wavelengths near 450 nm (Soret peak)

Meunier, B. *Chem. Revi* **2004**, *104*, 3947.

- The detailed mechanism of metabolism by CYPs has been described in the catalytic cycle



- There are more than 300 different CYP enzymes, which have been grouped into several families and subfamilies based on the amino-acid sequence.
- Out of these, 18 CYP families have been identified in mammals, comprising majorly of families CYP1, CYP2 and CYP3.
- Fraction of clinically used drugs (248 drugs) metabolized by P450 isoforms and factors influencing variability.



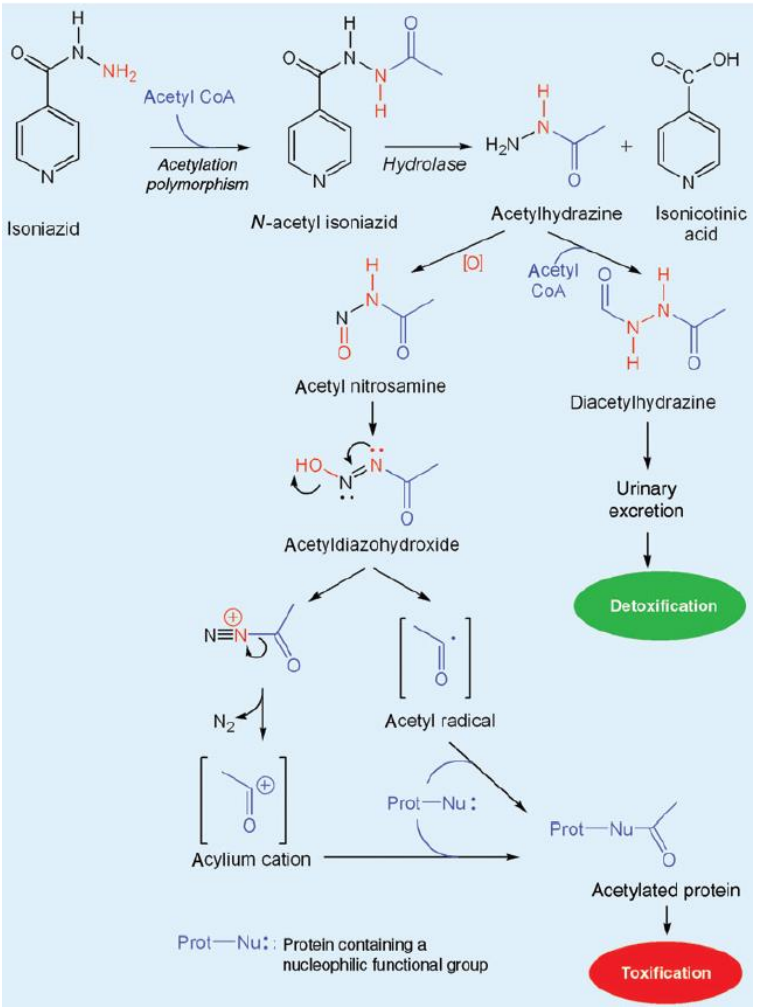
Zanger, U. M. *Pharmacol Thera* **2013**, *138*, 103.

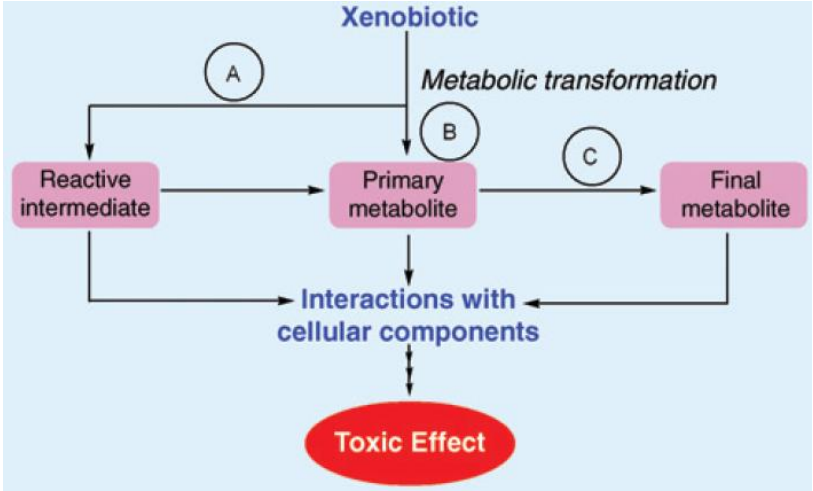
P450 enzyme classification:

- In human around 30 CYP enzymes which are responsible for drug metabolism.
- however, that 90% of drug oxidation can be attributed to six main enzymes: CYP 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4.
- CYP450 3A4 is involved in the metabolism of many different drugs (almost 50% of those on the market).
- The most significant CYP isoenzymes in terms of quantity are CYP3A4 and CYP2D6.
- CYP450 2E1 is involved in the oxidation of small molecules such as ethanol and chlorzoxazone.
- In humans - Central role in phase I drug metabolism
  - Significant problems in clinical pharmacology
  - Drug interactions

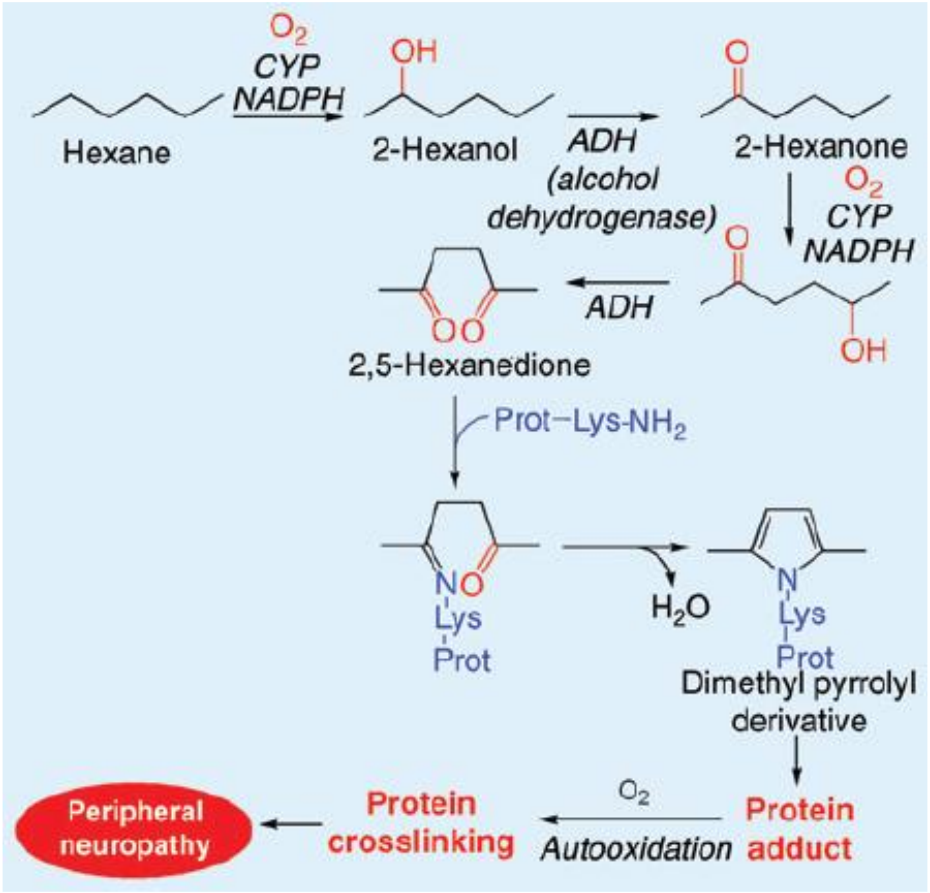
Biotransformations Leading to Toxic Metabolites: Chemical Aspect

- Differential metabolism of drugs, where one istoxic and the other non-toxic, representing structure–toxicity relationship.

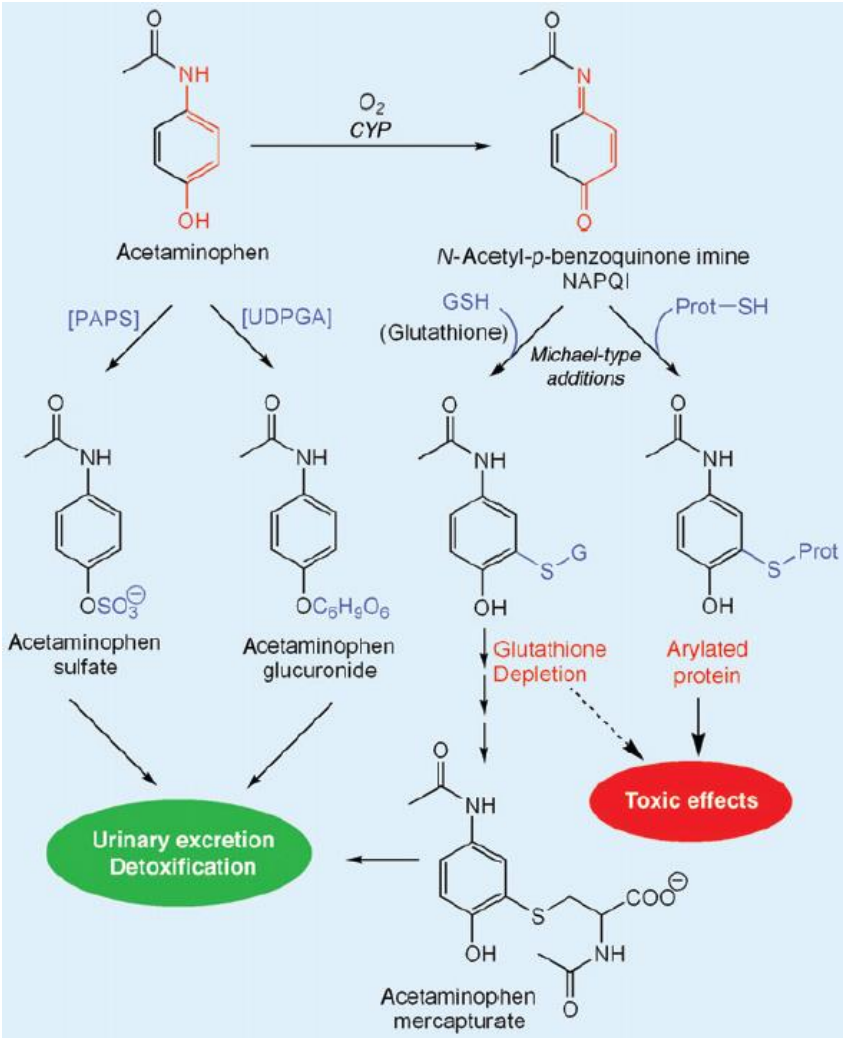




Biotransformations Leading to Toxic Metabolites: Chemical Aspect



Biotransformations Leading to Toxic Metabolites: Chemical Aspect



Background Information

- Hepatic metabolic stability is a key parameter in drug discovery.
- It is essential to identify metabolic liabilities early in drug discovery so they can be addressed during lead optimization.
- Both *in vitro* half-life ($t_{1/2}$), intrinsic clearance (CL_{int}) or compound hepatic clearance (CL_H) are utilized to express metabolic stability.
- Metabolic stability is typically first measured *in vitro* using liver microsomes and data from this assay is used to guide structural modifications to improve stability or select the best compounds for in vivo pharmacokinetic (PK) and efficacy testing.
- Liver microsomes are enriched with CYP450 enzymes, localized in the endoplasmic reticulum membrane, which are responsible for the metabolism of the majority (70–80%) of clinically approved drugs.
- The metabolic stability assays offer a method to calculate the rate of clearance of a test compound over time in microsomal incubations, and these data are used to evaluate intrinsic clearance.

benefits of using liver microsomes for drug metabolism studies:

- The liver is the main organ of drug metabolism in the body.
- Subcellular fractions such as liver microsomes are useful *in vitro* models of hepatic clearance as they contain many of the drug metabolising enzymes found in the liver.
- Microsomes are easy to prepare and can be stored for long periods of time.
- They are easily adaptable to high throughput screens which enable large numbers of compounds to be screened rapidly and inexpensively.

overview of microsomal stability assay:

- The microsomes are incubated with the test compound at 37°C in the presence of the co-factor, NADPH, which initiates the reaction.
- The reaction is terminated by the addition of 90% acetonitrile-water containing internal standard.
- Following centrifugation, the supernatant is analysed on the LC-MS/MS.
- The disappearance of test compound is monitored at 5-different time points over a 40 minute time period.
- An example of a typical depletion profile is shown in Figure below.
- The elimination constant (k_{el}), half-life ($t_{1/2}$) and intrinsic clearance (Cl_{int}) were determined in plot of ln(AUC) versus time, using linear regression analysis.

$$k_{el} = -slope$$

$$t_{1/2} = \frac{0.693}{k}$$

$$Cl_{\text{int}} = \frac{0.693}{t_{1/2}} \times \frac{\mu l_{incubation}}{mg_{microsomes}}$$

Interpretation of microsomal stability assay data:

- The test compounds can be classified in terms of their microsomal stability into low, medium and high clearance groups.
- The intrinsic clearance classification bands for mouse, rat, and human species are calculated according to the well stirred model equation:

$$\mathrm{CL_{int}} = \frac{CL_H}{fu \times (1 - E)}$$

where CL_H is a hepatic clearance (mL/min/kg), $CL_H = E \times Q_H$

Q_H = liver blood flow (mL/min/kg)

E = extraction ratio, assumed at 0.3 for low clearance and at 0.7 for high clearance compounds

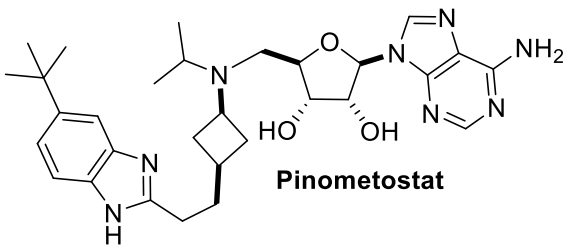
fu = fraction unbound in plasma, assumed at 1.

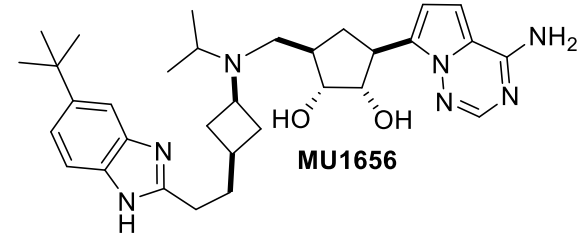
Table : classification bands typically used for categorising compounds into low, medium or high clearance.

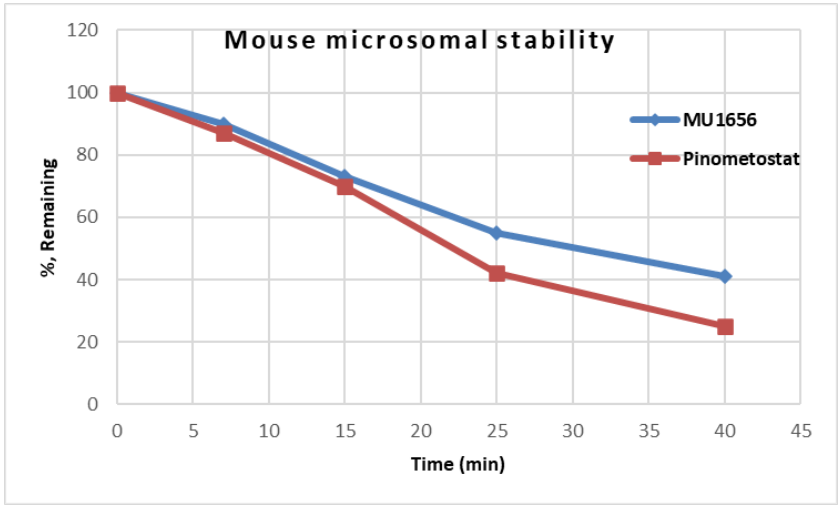
Classification group	Intrinsic clearance (µL/min/mg protein)		
	Mouse	Rat	Human
Low clearance	< 8.6	< 13	< 8.8
High clearance	> 48	> 72	> 48

Houston J.B. *Biochemical Pharmacology* **1994**, *47*, 1469; Barter Z.E. *Current Drug Metabolism* **2007**, *8*, 33; Iwatsubo T. *Journal of Pharmacology and Experimental Therapeutics* **1997**, *283*, 462.

Example of mouse microsomal stability for reference and test compound:





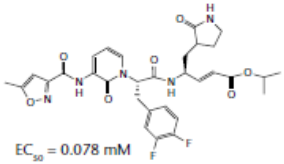
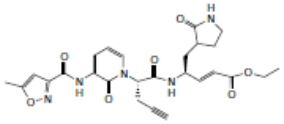
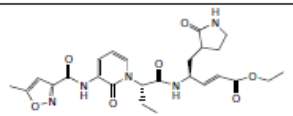
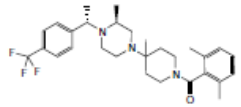
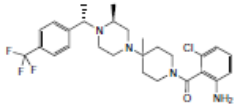
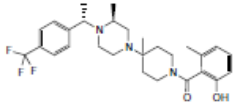
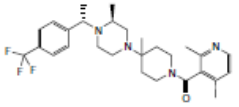
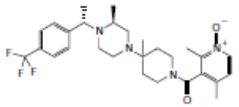


Compound ID	Incubation time (min)	Microsomal proteins (mg/ml)	Compound concentration (μM)	% Remaining	% Remaining without cofactor	R	k_{el} , (min^{-1})	$t_{1/2}$ min	Cl_{int} (μl/min/mg)
MU1656	40	0.415	2	41	112	0.997	0.023	29.8	56
Pinometostat	40	0.415	2	25	106	0.992	0.036	19.0	88

- **Strategies to enhance metabolic stability**

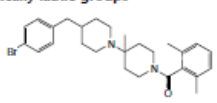
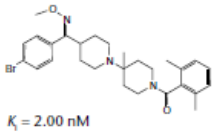
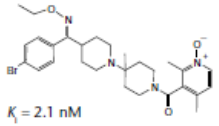
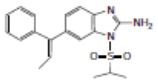
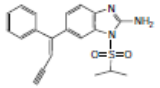
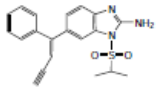
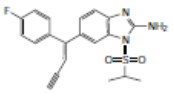
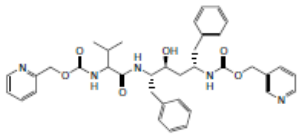
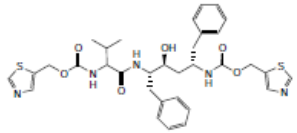
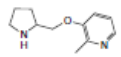
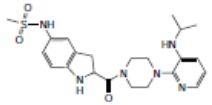
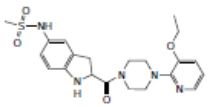
- Deactivation of aromatic rings to facilitate oxidation through substitution with strongly electron-withdrawing groups (e.g. CF_3, SO_2NH_2)
- Introduction of an *N*-t-butyl group to prevent *N*-dealkylation.
- Replacement of a labile ester linkage with an amide group.
- Constraining the molecule in a conformation that is unfavorable to the metabolic pathway, more typically, protecting the labile moiety by steric shielding.

Table 1. Enhancement of metabolic stability through structural modification

Approach and/or strategy	Enzyme or pathway	Lead compound	Optimized compound	Experimental model	Therapeutic class	Refs
Modification to improve lipophilicity and metabolic stability						
Reduce logP and logD						
	NA	EC_{50} = 0.078 mM clogP = 2.070 C 7 h = 0.012 μM	EC_{50} = 0.058 μM clogP = 0.180 C 7 h = 0.057 μM	Orally dosed monkey	3C protease inhibitor	[27]
	NA		 EC_{50} = 0.047 μM clogP = 0.66 C 7 h = 0.896 μM	Orally dosed monkey	3C protease inhibitor	[27]
Introduce isosteric atoms or polar functional group						
	NA	K_i = 1.000 nM $AUC_{0\text{-}6h}$ = 0.922 μg ml^{-1} h^{-1}	K_i = 5.000 nM $AUC_{0\text{-}6h}$ = 1.872 μg ml^{-1} h^{-1}	Orally dosed rat	CCR5 antagonists	[28]
	NA		 K_i = 5.000 nM $AUC_{0\text{-}6h}$ = 2.543 μg ml^{-1} h^{-1}	Orally dosed rat	CCR5 antagonists	[28]
	NA		 K_i = 2.300 nM $AUC_{0\text{-}6h}$ = 3.905 μg ml^{-1} h^{-1}	Orally dosed rat	CCR5 antagonists	[28]
	NA		 K_i = 7.000 nM $AUC_{0\text{-}6h}$ = 9.243 μg ml^{-1} h^{-1}	Orally dosed rat	CCR5 antagonists	[28]

Nassar, A. F. *Drug Discovery Today* **2004**, *9*, 1020.

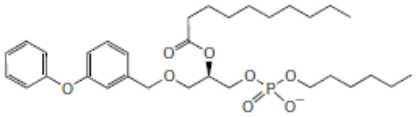
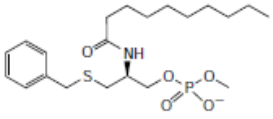
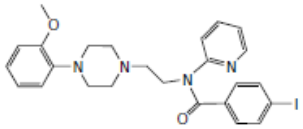
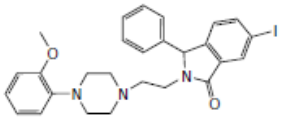
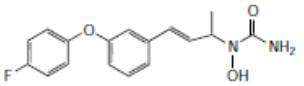
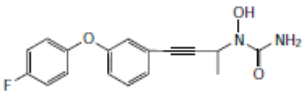
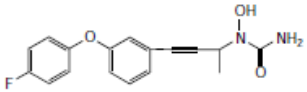
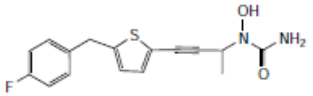
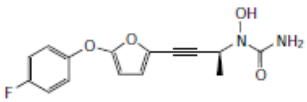
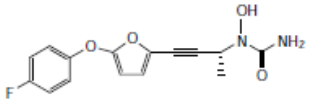
Modification of metabolically labile groups

Remove or block the vulnerable site of metabolism					
Benzylic oxidation	K_i = 66.00 nM AUC_{0-6h} = 0.04 μg ml^{-1} h^{-1}	K_i = 2.00 nM AUC_{0-6h} = 1.40 μg ml^{-1} h^{-1}	Orally dosed rat	CCR5 antagonists	[29]
					
		K_i = 2.1 nM AUC_{0-6h} = 6.5 μg ml^{-1} h^{-1}	Orally dosed rat	CCR5 antagonists	[29]
					
Allylic oxidation	IC_{50} = 0.06 μg ml^{-1} C_{max} = 14–140 ng ml^{-1}	IC_{50} = 0.02 μg ml^{-1} C_{max} = 70–300 ng ml^{-1}	Orally dosed monkey	Vinylacetylene antivirals	[30]
					
Phenyl oxidation	IC_{50} = 0.02 μg ml^{-1} %F = 9.00	IC_{50} = 0.04 μg ml^{-1} %F = 23.00	Orally dosed monkey	Vinylacetylene antivirals	[30]
					
N-oxidation	AUC = 1.98 μg ml^{-1} h^{-1} %F = 26.00	AUC = 4.24 μg ml^{-1} h^{-1} %F = 47.00	Orally dosed rat	HIV protease inhibitors	[31]
					
N-demethylation	$t_{1/2}$ = 3.0 h %F = 1.2	$t_{1/2}$ = 24.0 h %F = 61.5	Dog liver slices	nAChR ligands	[32]
					
N-dealkylation	$t_{1/2}$ = 10.8 min	$t_{1/2}$ = 46.8 min	Rat liver microsomes	BHAP reverse transcriptase inhibitors	[33]

Continued on following page

Nassar, A. F. *Drug Discov Today* **2004**, *9*, 1020.

Table 1. (continued)

Ester hydrolysis	 $t_{1/2}$ = 33 min C_{max} = 465 ng ml^{-1} %F = 4	 $t_{1/2}$ = 39 min C_{max} = 3261 ng ml^{-1} %F = 90	Rat blood and plasma, liver microsomes and homogenate	Hospholipase A inhibitors [34]
Amide hydrolysis	 K_i = 0.200 nM 40% and >60% degradation in human liver cytosol and microsomes, respectively	 K_i = 0.069 nM 10% and <5% degradation in human liver cytosol and microsomes, respectively	Human liver cytosol and microsomes	5-HT$_{1A}$ receptor ligand [35]
Glucuronidation (effect of linker)	 UDPGA rate = 0.19 nM, min^{-1} mg^{-1} of protein $t_{1/2}$ = 4.70 h	 UDPGA rate = 0.05 nM, min^{-1} mg^{-1} of protein $t_{1/2}$ = 5.50 h	Monkey liver microsomes and plasma	5-LO inhibitors [36]
Glucuronidation (effect of template)	 UDPGA rate = 0.050 nM, min^{-1} mg^{-1} of protein $t_{1/2}$ = 5.500 h	 UDPGA rate = 0.012 nM min^{-1} mg^{-1} of protein $t_{1/2}$ = 14.500 h	Monkey liver microsomes and plasma	5-LO inhibitors [36]
Glucuronidation (effect of stereochemistry)	 UDPGA rate = 0.02 nM, min^{-1} mg^{-1} of protein $t_{1/2}$ = 7.70 h	 UDPGA rate = 0.01 nM, min^{-1} mg^{-1} of protein $t_{1/2}$ = 8.70 h	Monkey liver microsomes and plasma	5-LO inhibitors [36]

Nassar, A. F. *Drug Discovery Today* **2004**, *9*, 1020.

improvement of metabolic stability via incorporation of Fluorine

- the relatively small size of the fluorine atom (vander Waals radius of 1.47 A°), comparable to hydrogen (van der Waals radius of 1.20 A°).
- the highly electron withdrawing property of fluorine.
- greater stability of the C–F bond compared to the C–H bond.
- greater lipophilicity of fluorine compared to hydrogen.

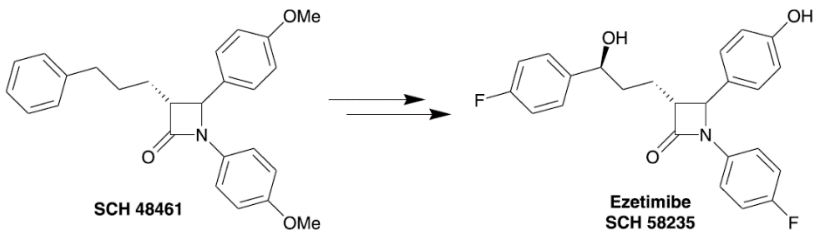
Insertion of fluorine atoms into molecules can

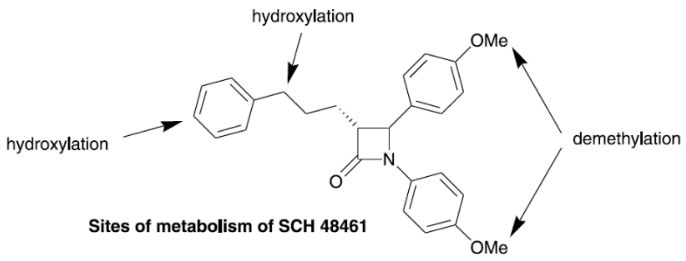
- Improved metabolic stability
- Altered physicochemical properties
- Increased binding affinity

Example : Ezetimibe (SCH 58235)

- approved in 2002 by the FDA to reduce cholesterol levels in patients with hypercholesterolaemia.

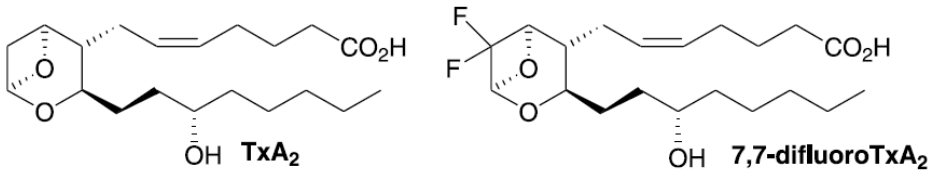
- developed from the compound SCH 48461, which is susceptible to metabolic attack in four primary sites via demethylation, hydroxylation, and/or oxidation.



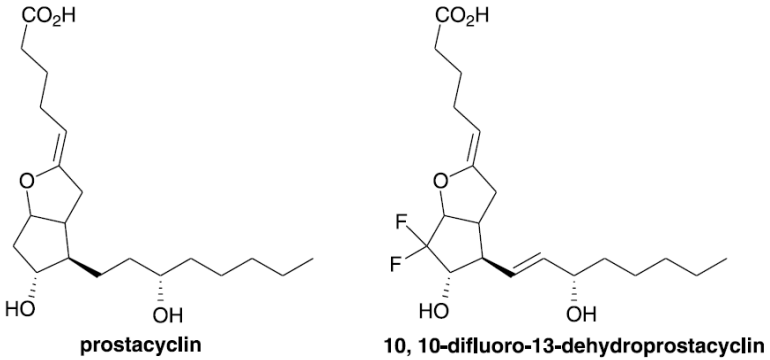


Fluorine substitution to extend the biological half life

- The prostanoids, short half-lives *in vivo* of less than 5 minutes.
- The platelet-aggregating agent, thromboxane A2 (**TxA2**) has an unusual oxetane acetal structure that undergoes hydrolytic cleavage at pH 7.4 and has a half-life of around 30 seconds.
- By introducing fluorine into the oxetane ring (**7,7-difluoro-TxA2**) the rate of hydrolysis is 108-fold slower than **TxA2**.

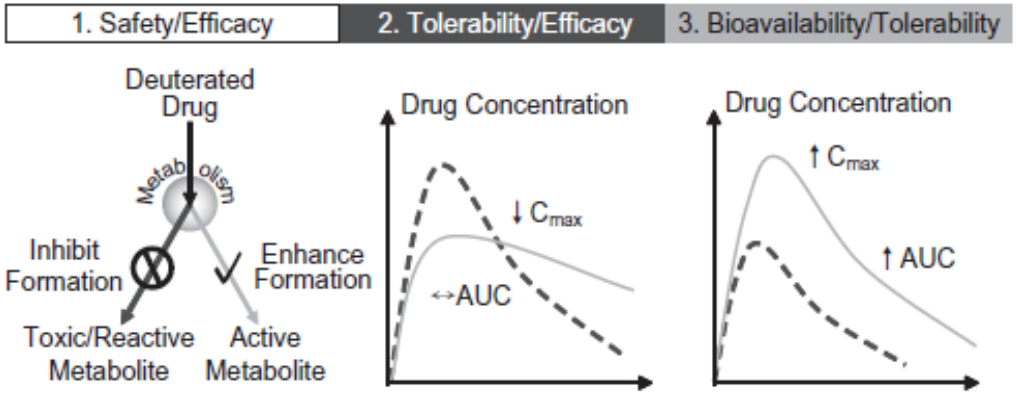


- Prostacyclin an inhibitor of platelet aggregation contains an acid labile enol-ether group that is responsible for its short half-life.
- By introducing a fluorine atom a to the enol-ether group, the electron density on the enol-ether group is reduced via its inductive effects, which improves the stability of the molecule to acid hydrolysis.



improvement of metabolic stability via incorporation of Deuterium

- Selective replacement of hydrogen with deuterium leads to increased bond strength which in turn increases the biological half-life and thus metabolic stability of the drug.

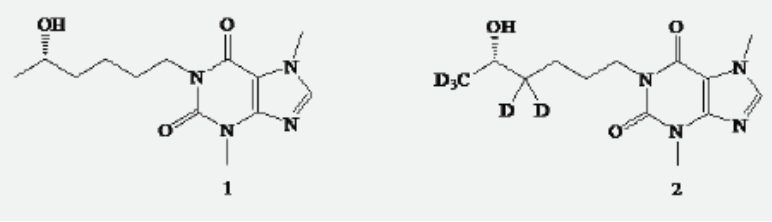


- Metabolic shunting resulting in reduced exposure to undesirable metabolites or increased exposure to desired active metabolites.
- Reduced systemic clearance resulting in increased half-life.
- Decreased pre-systemic metabolism resulting in higher bioavailability of unmetabolized drug.

Harbeson, S. *Med-Chem News* ***2014,*** *24,* 8. Sukhninder, K. Glob J Pharma Sci. **2017**, 1, 555566.

1. CTP-499: A deuterated pentoxifylline (Trental)

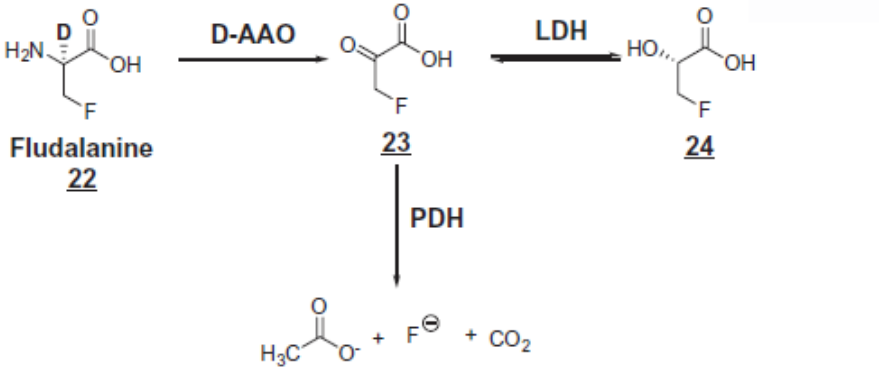
- Pentoxifylline, an active metabolite of Trental, is an effective agent for the treatment of nephropathy in Type-II diabetic patients.
- It is significantly metabolized to HDX **1**.
- The deuterated version of **1**, i.e., CTP-499 (**2**) has showed enhanced metabolic profile of the compound.
- Phase II study showed a delay of end stage of renal failure, suggesting CTP-499 can be used for type 2 diabetes and chronic kidney disease.



Sabounjian, L. *Clin Pharmacol Drug Dev* **2016**, *5,* 314.

2. Fludalanine

- developed by Merck, one of the earliest deuterated drug candidates to enter the clinic.
- The combination of **22** with cycloserine provides a broadspectrum and potent antibacterial.
- The hydrogen analog is also an effective antibacterial, but preclinical studies reportedly demonstrated that **23** was metabolized to form 3-fluorolactate **24** a toxin that caused brain vacuolization, which is in equilibrium with 3-fluoropyruvate **23** during metabolism.
- The deuterated analog **22** showed reduced level of 3-fluorolactate **24** production in healthy volunteers.
- However, higher levels of 3-fluorolactate **24** were observed in patients; therefore, studies on fludalanine were discontinued at Phase IIb.

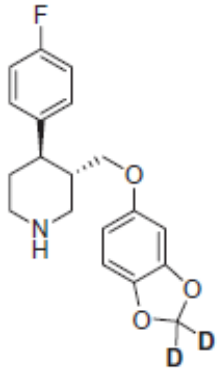


Scheme 6. Proposed metabolism of fludalanine (**22**) to produce L-fluorolactate (**24**). D-amino acid oxidase (D-AAO); lactate dehydrogenase (LDH), and pyruvate dehydrogenase (PDH).

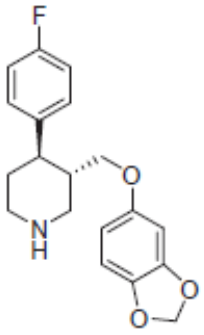
Kahan, F. *Chem Engin News* **2009,** 87.

3. CTP-347

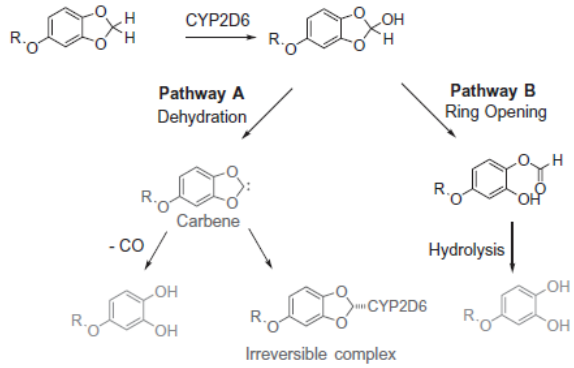
- CTP-347, a selectively deuterated analogue of paroxetine a centrally acting SSRI (selective serotonin reuptake inhibitor) for the treatment of major depressive disorder, panic disorder, social anxiety disorder.
- Low doses of **26** have been reported to have good efficacy in treating hot flashes.
- However, **26** is not only metabolized by CYP2D6 but also potently inhibits its own metabolism by irreversibly inactivating that enzyme.
- Its use, therefore, can be complicated in patients potentially benefiting from this agent due to possible drug-drug interactions with other drugs metabolized by CYP2D6. In the case of thioridazine, coadministration of paroxetine is contraindicated.



CTP-347
25



Paroxetine
26

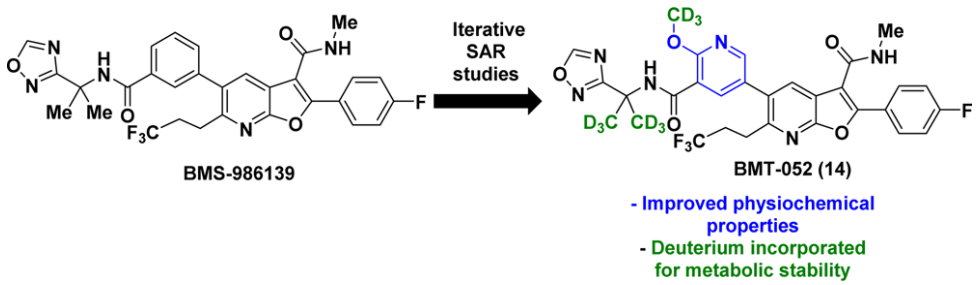


Scheme 7. Proposed inactivation pathway for CYP2D6 by a paroxetine metabolite. Pathway A produces a putative reactive metabolite that results in inactivated enzyme. Pathway B rapidly cleared catechol metabolite via formate ester hydrolysis. Some of the carbene metabolite may also convert to the catechol via decarbonylation.

Uttamsingh V. *J. Pharmacol. Exp. Ther* **2015**, *354*, 43.

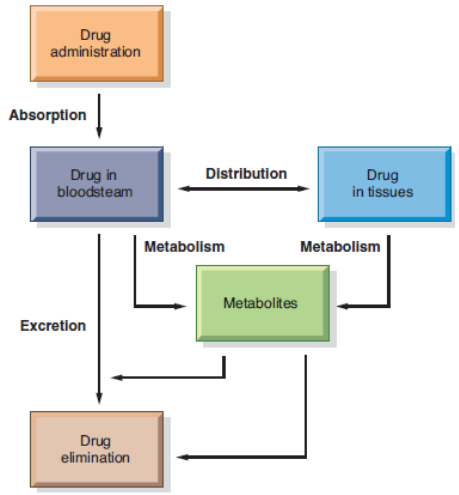
4. BMT-052

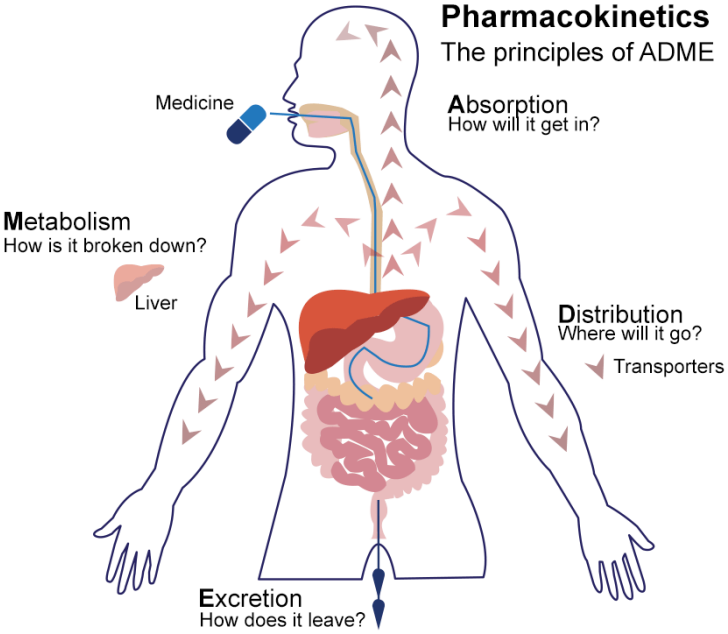
- the development of second generation pan-genotypic inhibitor BMS-986139 halted due to unexpected microcrystallization in multiple tissues at elevated doses in both rats and dogs in investigational new drug (IND) toxicology studies.
- through iterative SAR studies and systematically incorporating deuterium into both the C5 and amide substituents, the promising preclinical compound 14 was identified.
- Compound 14 exhibited low clearance (Cl), a moderate volume of distribution (Vss), and good oral bioavailability (F%) across the species.
- Compound 14 had an improved solubility and overcomes the micro crystallization problems observed during the IND toxicology studies of BMS-986139.



Parcella, K. *ACS Med. Chem. Lett.* **2017**, *8*, *771.*

- defined as the study of the quantitative relationship between administered doses of a drug and the observed plasma/blood or tissue concentrations.
- pharmacokinetics is concerned with the drug ADME - absorption, distribution, metabolism, and excretion or elimination.
- When should they be determined? – early in the process

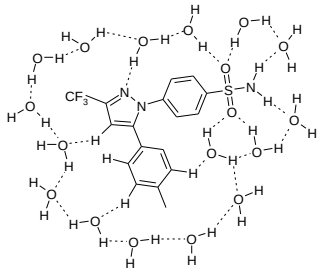




- **Drug delivery routes**

1. **Enteral routes** : delivers the compound into the body through the gastrointestinal (GI) tract.
   - oral (PO), sublingual, rectal.
2. **Parental routes** : administration can be performed by injection.
   - intramuscular (IM), subcutaneous (SC) and intravenous (IV) etc.

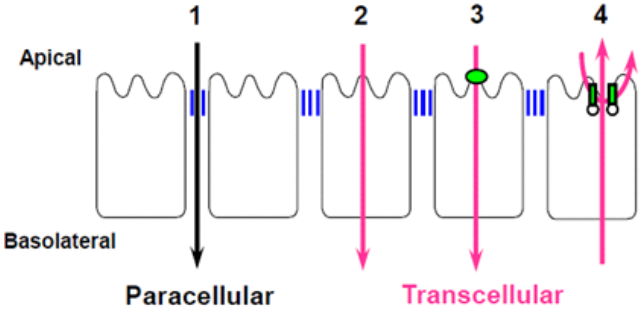
Absorption (will my drug get into the body??)



- To be in solution compound must be fully hydrated (surrounded by water).
- The greasier your compound, the less it likes to be hydrated.
- The more polar, the more aqueous soluble.

Absorption pathways

- Most compounds absorbed by transcellular route.
- It is possible for molecules to be absorbed between the cells of the gut wall (paracellular absorption), but this is relatively rare.



1-2: passive diffusion
3: active transport
4: efflux (Pgp)

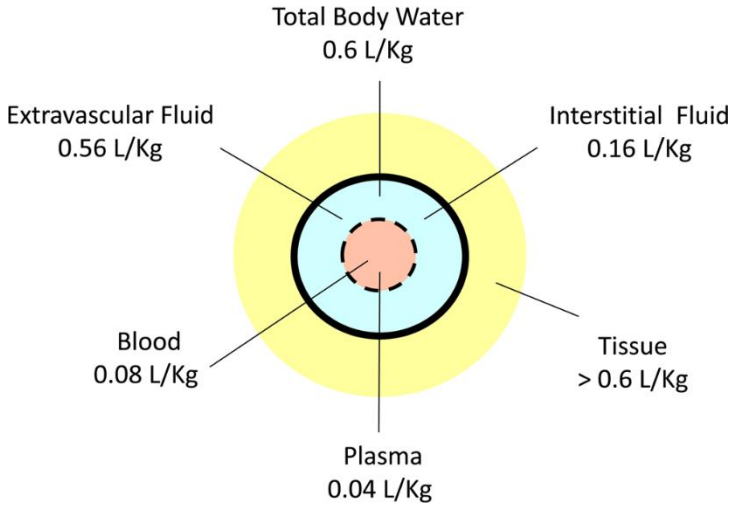
Pgp

Pharmacokinetic parameters:

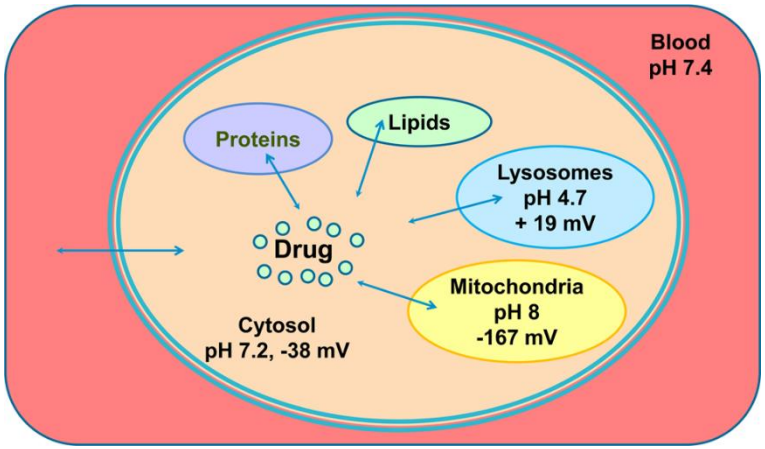
1. Volume of distribution (Vd)
2. Clearance
3. half-Life
4. Bioavailability
5. Bioequivalence (BE)

Volume of distribution

- pharmacokinetic parameter representing an individual drug's propensity to either remain in the plasma or redistribute to other tissue compartments.
- Vd is a proportionality constant that relates the total amount of drug in the body to the plasma concentration of the drug at a given time.
- Volume of Distribution (L) = Amount of drug in the body (mg) / Plasma concentration of drug (mg/L).
- It is a major determinant of half-life and dosing frequency of a drug.
- The volume of distribution values for small molecule drugs should be compared to the physiological tissue volumes.



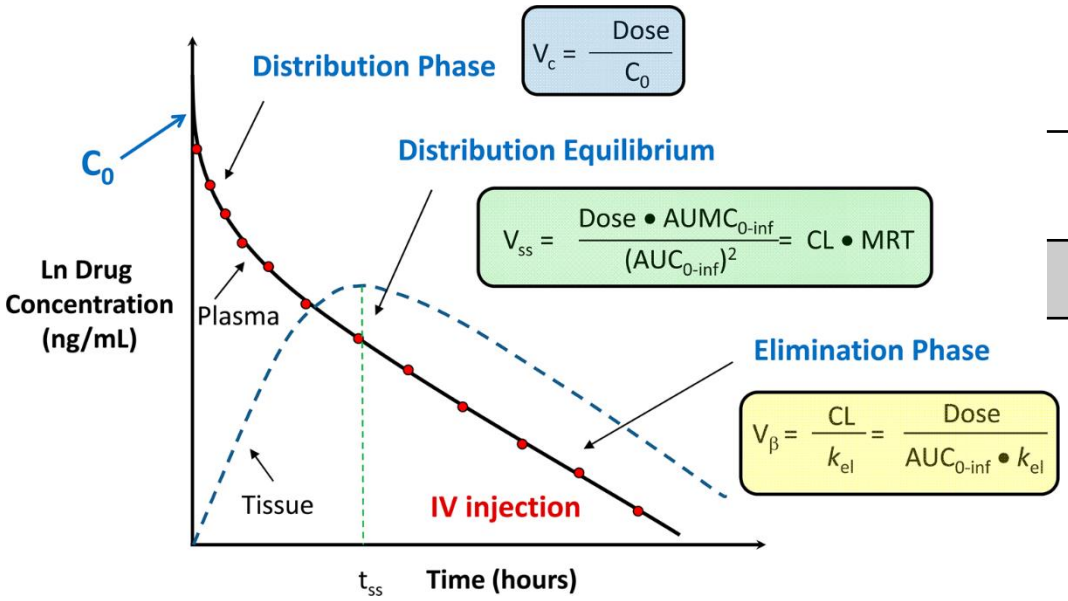
Physiological volumes of body fluids in humans.



Smith, D. A. *J. Med. Chem.* **2015**, *58*, 5691.

Volume of distribution

- the most calculated parameter is volume of distribution at steady state (V_{ss}).
- it represents the apparent distribution volume associated with the steady-state dosing paradigm under which most drugs are developed.
- the volume of the central compartment (Vc) represents the apparent volume of distribution immediately following an intravenous bolus dose.
- V_β can be derived from the terminal elimination phase half-life.
- The volume of distribution values for small molecule drugs should be compared to the physiological tissue volumes.



Definition of the different volume of distribution terms: V_c, V_{ss}, and V_β.

Classification of steady state volume of distribution (V_{ss}, L/kg).

species	low	moderate	high	very high
all	<0.6	0.6–5	5–100	>100

Smith, D. A. *J. Med. Chem.* **2015**, *58*, 5691.

- V_{ss} affects half-life and duration of action. For compounds with equal daily dose, the compound with lower V_{ss} (shorter half-life) may need to be dosed more frequently (at lower individual doses) to achieve a similar pharmacodynamic profile as the one with higher V_{ss}.
- Large V_{ss} does not indicate that drugs will reach the remote sites of action and be pharmacologically active. Similarly, low V_{ss} does not mean that the compound will not reach the remote site of action.
- High V_{ss} and high total tissue concentration do not necessarily translate to better activity for disease targets in the tissues.
- Transporters, pH gradients, and electrochemical potential scan impact V_{ss}, but the magnitude is usually small. Binding typically plays a dominant role in determining V_{ss}.
- Structure modification can be applied to increase/decrease V_{ss} and duration of action while maintaining clearance. Achieving this is not straightforward and requires well-directed strategies and design.
- The major approaches to modulate V_{ss} are introducing basic functional groups and increasing lipophilicity in a way that does not increase unbound intrinsic clearance.
- Acidic compounds will need to have very low intrinsic clearance to have long duration of action because their V_{ss} values are typically small.

Smith, D. A. *J. Med. Chem.* **2015**, *58*, 5691.

- It quantitates the irreversible removal of a drug from the measured matrix (blood or plasma).
- clearance parameter (*CL*):

  rate of elimination = *CL* × concentration
- Units: *CL* = volume/time.

- Clearance = $\frac{\text{rate at which a drug is removed from plasma (mg/min)}}{\text{concentration of that drug in the plasma (mg/mL)}}$

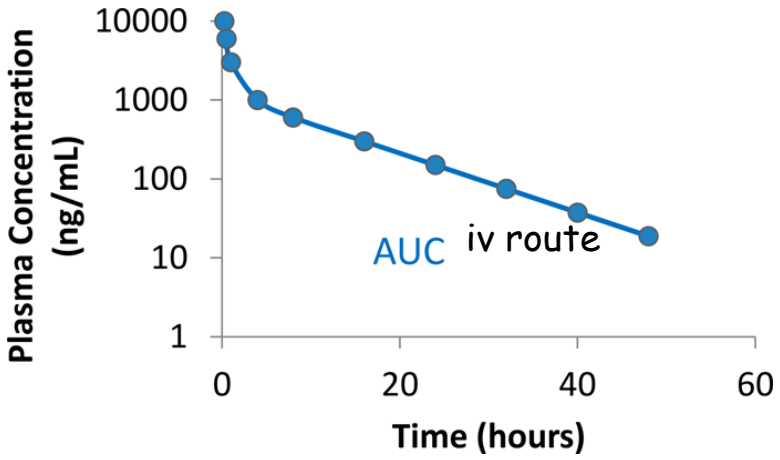
- While *CL* is typically constant, the rate of drug elimination is concentration dependent.
- measurement of the concentration of drug in plasma over a time course, the area under the plasma concentration versus time course (*AUC*) can be calculated.

  CL = dose/AUC
- The relationship between CL_b and CL_p is: $\mathbf{CL_b = CL_p/R_b}$

  where CL_b = blood clearance

  CL_p = plasma clearance

  R_b = blood-to-plasma ratio

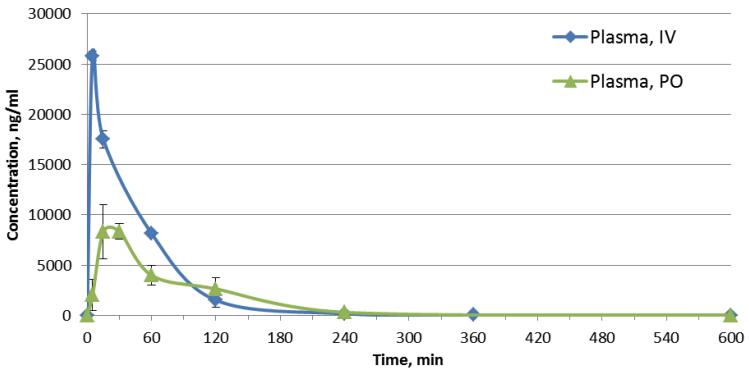


Smith, D. A. *J. Med. Chem.* **2019**, *62*, 2245.

Introduction:

- The route of drug administration is critical for clearance assessment.
- iv route : all of the administered dose reaches the systemic circulation (blood).
- po route: potential barriers, where the dose is applied to the gastrointestinal (gi) tract and has to be absorbed and avoid first pass extraction by the gut wall and liver prior to reaching the systemic circulation.
- The AUC following po is often lower than that following iv of the same dose due to incomplete absorption, first-pass intestinal extraction, and/or hepatic extraction.
- Clearance following po (CL_{po}) is most often higher than CL_s.
- The
- relationship between oral clearance and systemic clearance relies on an understanding of oral bioavailability (F):

$$CL_{po} = CL_s/F$$



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Importance of clearance in drug disposition:

(a) Effect of clearance on half-life:

- Together with volume of distribution (V_d), clearance governs the elimination rate (k_{el}) of a drug, and ultimately half-life ($t_{1/2}$).
- approximation of half-life from V_d and *CL*:

$$CL \cong V_d \times k_{el}$$

$$t_{1/2} \cong \ln 2 \times V_d/CL$$

(b) Effect of clearance on oral bioavailability:

- During absorption, drugs are exposed with metabolizing enzymes in the GI tract.
- the liver can also extract a portion of the administered dose by metabolism and/or transport due to the fact that drugs directly enter the liver via the hepatic portal vein before reaching systemic circulation. This process is termed **hepatic first-pass extraction**.
- the hepatic extraction is a key component of both oral bioavailability and clearance.
- minimizing hepatic extraction by lowering clearance is a key component in optimizing oral bioavailability.

$$F = F_a \times F_g \times (1 - E_h)$$

where, F = fraction bioavailable, F_a = fraction absorbed

$E_h = CL/Q$

F_g = fraction escaping gut wall extraction

E_h = hepatic extraction ratio, Q = hepatic blood flow

Smith, D. A. *J. Med. Chem.* **2019**, *62*, 2245.

(c) Effect of clearance on efficacious dose.:

- The importance of clearance in the relationship between steady state concentration and dose was recognized early by Wagner in 1965.
- modulation of clearance has a direct effect on efficacious dose within a particular chemical series.

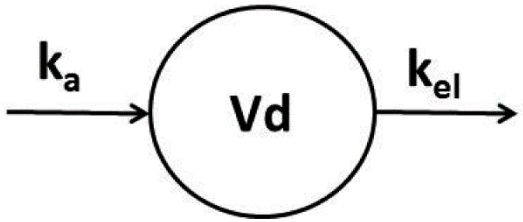
$$\text{dose} = C_{ss,av} \times CL \times \tau/F$$

where $C_{ss,av}$ = average concentration at steady state (related to potency and target concentration)

τ = dose interval.

Smith, D. A. *J. Med. Chem.* **2019**, *62,* 2245.

- **Relevance of half-life ($t_{1/2}$) in drug design:**
- $t_{1/2}$ is defined as the time required for the concentration of a drug (typically in blood or plasma) to reduce to half of its initial value when the concentrations of the drug are in simple exponential (log–linear) decline.
- A half-life of 12–48 h is generally ideal for once daily dosing of oral drugs.
- If the half-life is too short, it may require more frequent dosing in order to maintain desired exposures and avoid unnecessarily high peak concentrations.
- When considering a design effort to optimize $t_{1/2}$, there are several key questions that need to be understood, namely:
  - What is the optimal $t_{1/2}$ to support once daily administration?
  - How can $t_{1/2}$ be modulated?
  - What are the important considerations in measuring $t_{1/2}$ during compound optimization?



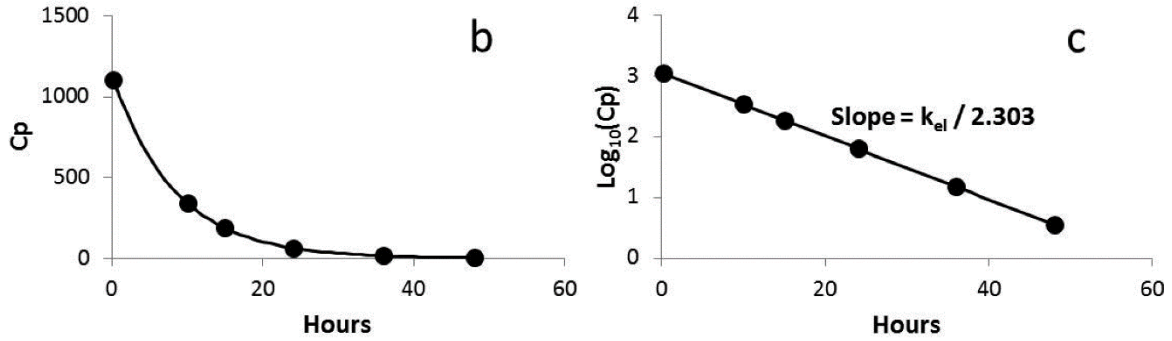
first order absorption and elimination rates.

K_a = first order rate constant of absorption.

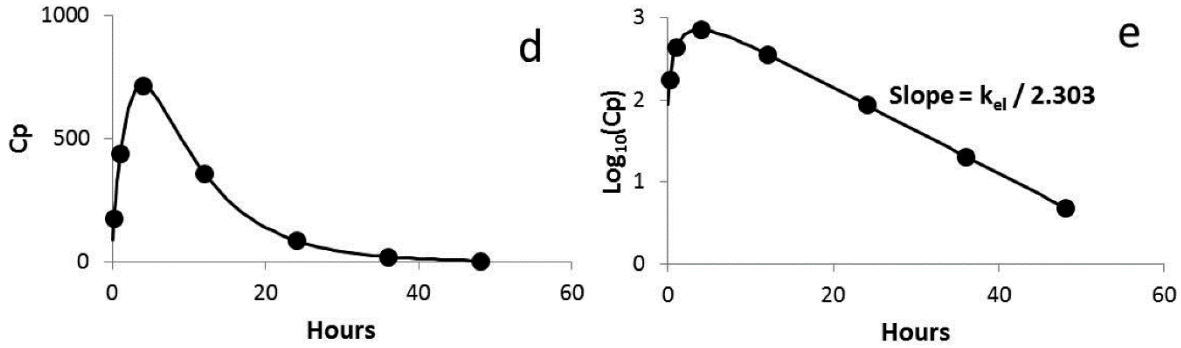
K_{el} = first order rate constant of elimination.

Smith, D. A. *J. Med. Chem.* **2018**, *61,* 4273; Gunaydin, H. *ACS Med. Chem. Lett.* **2018**, *9*, 528.

- the characteristic exponential decline following iv bolus administrations.



- absorptive phase followed by monoexponential decline following oral administration.



- Drug is eliminated via another first order rate constant (k_{el}) which determines the $t_{1/2}$ of the drug under conditions in which the rate of absorption is more rapid than the rate of elimination.
- k_{el} can be estimated from the slope obtained after plotting the log_{10} of concentration versus time.
- The k_{el} parameter determines the $t_{1/2}$ of a drug according to :

$$t_{1/2} = \ln(2)/k_{el}$$

Smith, D. A. *J. Med. Chem.* **2018**, *61,* 4273; Gunaydin, H. *ACS Med. Chem. Lett.* **2018**, *9*, 528.

relation between half-life, volume of distribution and clearance:

$$t_{1/2} = \ln(2)V_{ss}/CL$$

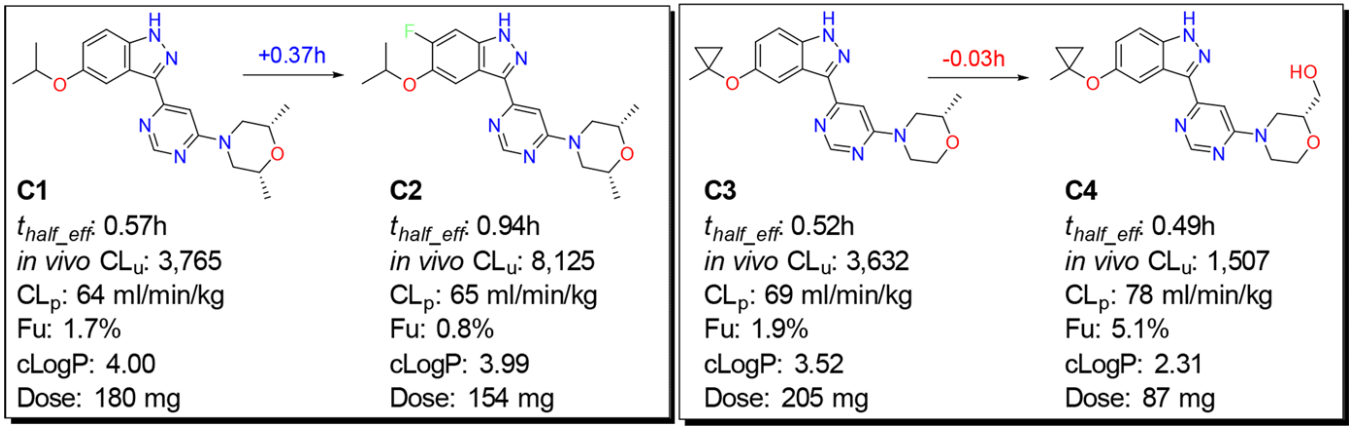
- $t_{1/2}$ will be proportional to volume and inversely proportional to clearance.

- **How can $t_{1/2}$ be modulated?**
- reducing plasma CL (e.g., increase metabolic stability, reduce renal clearance, reduce hepatic uptake by active transporters and biliary clearance) and/or second increasing V_{ss}.
- modulation of plasma protein binding in the absence of changing CL or V_{ss} will not affect $t_{1/2}$.
- changing fraction unbound in plasma will often change CL and V_{ss} in equal and opposite directions.
- it is also possible to improve the $t_{1/2}$ by modifying the delivery of the compound (formulation) depending upon aqueous solubility, high membrane permeability, and low efflux transport.

Smith, D. A. *J. Med. Chem.* **2018**, *61,* 4273; Gunaydin, H. *ACS Med. Chem. Lett.* **2018**, *9*, 528.

Matched molecular pairs (MMP):

- are pairs of molecules that differ by a single chemical transformation and are used to relate the changes in measured activities to changes in chemical structures.
- Examples of MMPs that show the change in half-lives upon changing the lipophilicity of the molecules.

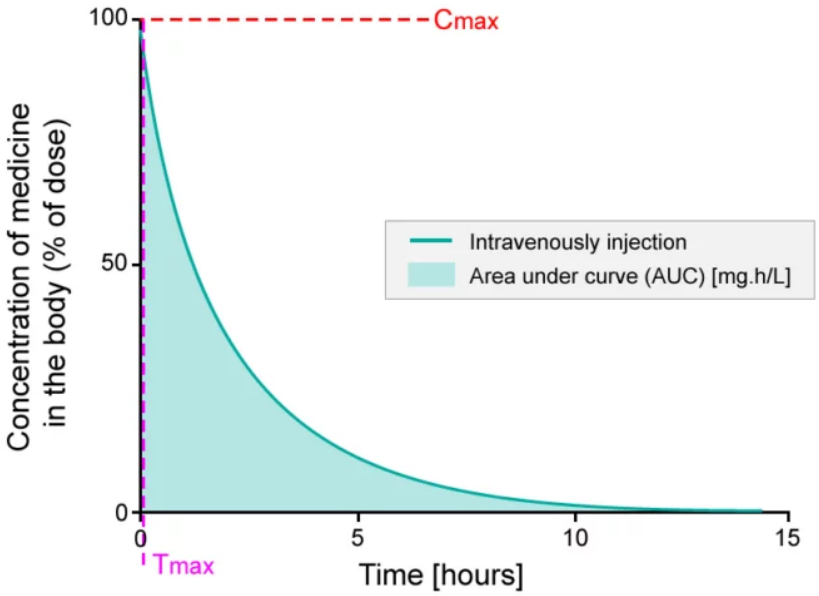


Smith, D. A. *J. Med. Chem.* **2018**, *61,* 4273; Gunaydin, H. *ACS Med. Chem. Lett.* **2018**, *9*, 528.

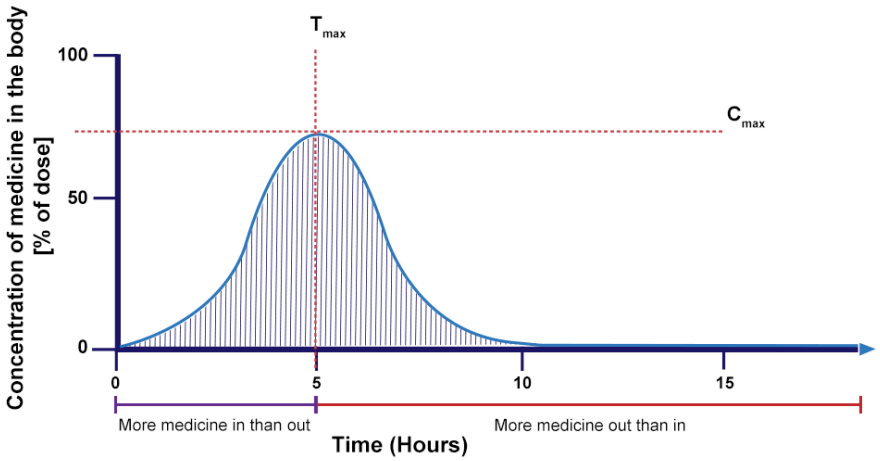
- the fraction (percentage) of an administered drug that reaches the systemic circulation (blood).
- The route of administration and the dose of a drug have a significant impact on both the rate and extent of bioavailability.
- to have a therapeutic effect, the active substance
  - has to enter the body.
  - needs to be available in the correct dose at the specific site (target site).
  - needs to reach the target site within a certain time.
  - needs to available there for a defined time.

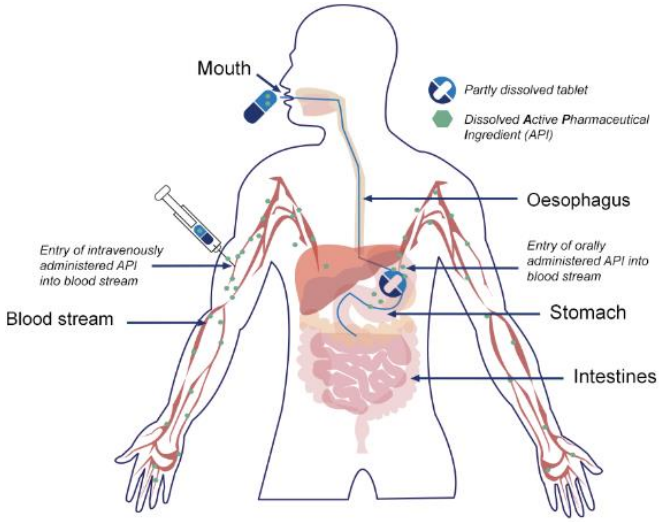
- When giving an injection directly into the bloodstream, i.e. an intravenous injection (IV), the bioavailability is defined as 100%.

- Tmax = time where the highest concentration of the medicine is found in the blood.
- Cmax = maximum concentration of the medicine found in the blood.



Oral bioavailability:



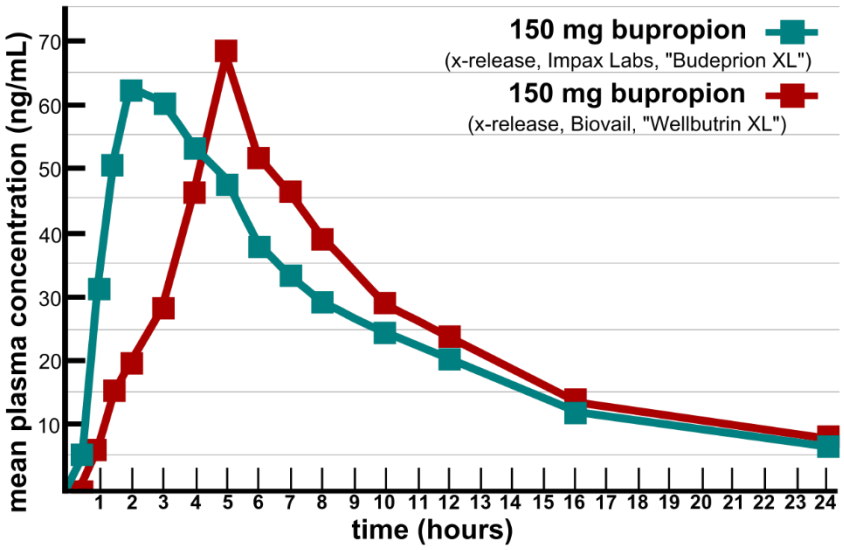


- Lower bioavailability can be the result of poor or no absorption from the stomach and the intestines.
- When the active substance is absorbed, it reaches the hepatic portal vein first, and is transported to the liver. This is the first time the active substance is metabolised in the liver, referred to as the '**first pass metabolism**'.
- The non-metabolised part of the active substance, normally less than 100%, will reach systematic circulation via the hepatic vein. The amount that actually reaches systemic circulation is referred to as the '**absolute bioavailability**'.

- the property wherein two drugs with identical active ingredients or two different dosage forms of the same drug possess similar bioavailability and produce the same effect at the site of physiological activity.
- If two drugs are bioequivalent, there is no clinically significant difference in their bioavailability.
- Bioequivalence implies that different drugs release their active ingredient in the equivalent dose, rate of absorption, and quality.

- bioequivalence studies are also performed for brand name drugs in some situations such as:

- early and late clinical trial formulations.
- between the formulations used in clinical trials and the product to be marketed for new medicines.
- changes in formulation have occurred after a brand name drug has been approved.



A bioequivalency profile comparison of 150 mg extended-release bupropion as produced by Impax Laboratories for Teva and Biovail for GlaxoSmithKline.

Why genotoxicity assessment of drugs?

- for safety assessment of drugs/products.

General purpose:

- detect compounds which induce genetic damage directly or indirectly by various mechanisms.
- positive compounds may induce cancer and/or heritable defects.

A standard genotoxicity testing includes:

- AMES test – a test for gene mutations in bacteria
- micronucleus test - chromosomal fragment in cytoplasma of erythrocytes or binuclear cells, lymphocytes.
- Cytogenetic analysis of bone marrow cells of experimental animals or of human peripheral lymphocytes - chromosomal breaks or rearrangements.
- Comet assay – single strand DNA breaks.

Ames test:

- Ames test is developed by Bruce N. Ames in 1970s to test for determining if the chemical is mutagens.
- based on the principle of reverse mutation or back mutation. So, the test is also known as bacterial reverse mutation assay.
- can detects suitable mutants in large population of bacteria with high sensitivity.

Test organism:

- Ames test uses several strains of bacteria (Salmonella, E.coli) that carry mutation.

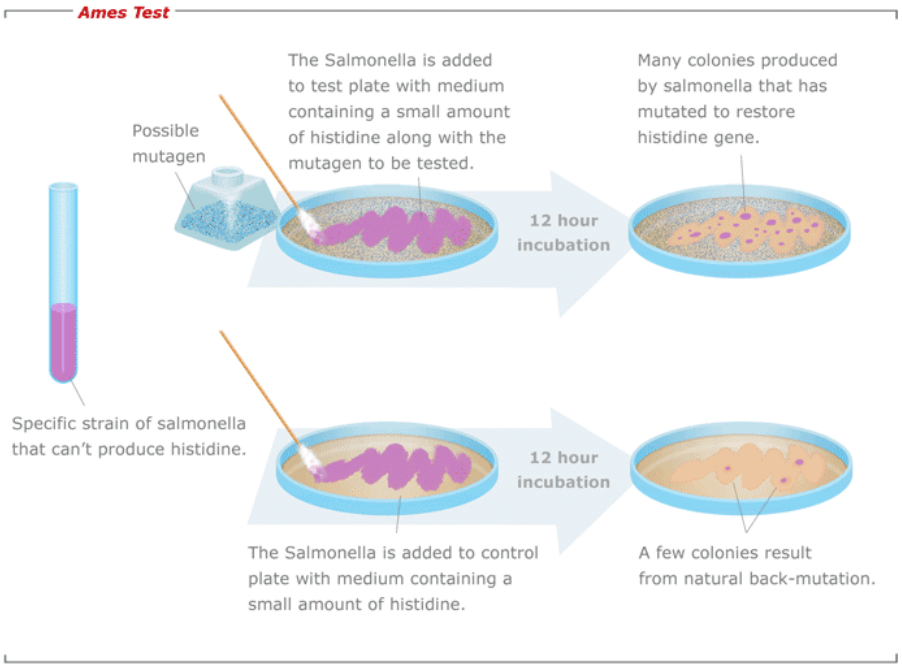
e.g. - A particular strain of *Salmonella Typhimurium* carry mutation in gene that encodes histidine.

- So it is an auxotrophic mutant which loss the ability to synthesize histidine utilizing the ingredients of culture media. Those strains are known as **His-** and require histidine in growth media.
- Culturing **His-** salmonella is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine (**His+**).
- This is the reverse mutation. Such chemicals responsible to revert the mutation is actually a mutagen. So, this Ames test is used to test mutagenic ability of varieties of chemicals.

Mortelmans, K. *Mutation Research* **2000**, *455*, 29.

General procedure:

- The bacteria are spread on an agar plate with small amount of histidine.
- This small amount of histidine in the growth medium allows the bacteria to grow for an initial time and have the opportunity to mutate.
- When the histidine is depleted only bacteria that have mutated to gain the ability to produce its own histidine will survive.
- The plate is incubated for 48 hours.
- The mutagenicity of a substance is proportional to the number of colonies observed.



Limitations:

- Salmonella typhimurium is a prokaryote, therefore it is not a perfect model for humans.

- Rat liver S9 fraction is used to mimic the mammalian metabolic conditions so that the mutagenic potential of metabolites formed by a parent molecule in the hepatic system can be assessed; however, there are differences in metabolism between humans and rats that can affect the mutagenicity of the chemicals being tested.

- the use of human liver S9 fraction; its use was previously limited by its availability, but it is now available commercially and therefore may be more feasible.

- Mutagens identified in the Ames test need not necessarily be carcinogenic, and further tests are required for any potential carcinogen identified in the test.

  - e.g. drugs that contain the nitrate moiety sometimes come back positive for Ames when they are indeed safe. The nitrate compounds may generate nitric oxide, an important signal molecule that can give a false positive.

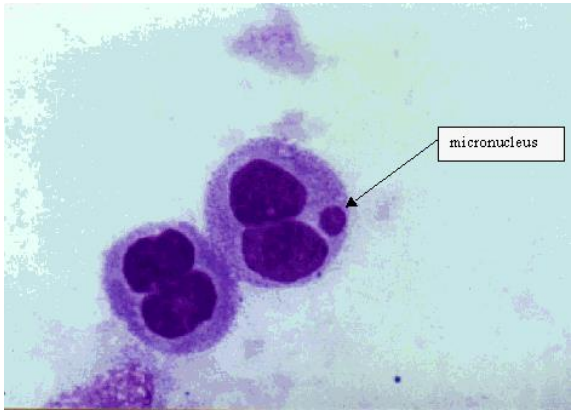
Genotoxicity Testing

Micronucleus test:

- micronucleus formation resulting from agents that cause chromosomal damage is a hallmark of genotoxicity.
- to determine if a compound is genotoxic to cells in culture by evaluating the presence of micronuclei.
- simple, reliable and reproducible.
- performed in erythrocytes generally, but currently its use has been extended to other tissues like liver, lung, skin etc.
- A mutagenic effect of tested compound is presented by increased number of micronuclei in 1000 of cells in comparison with control.
- There are two major versions of this test, in vivo and in vitro.

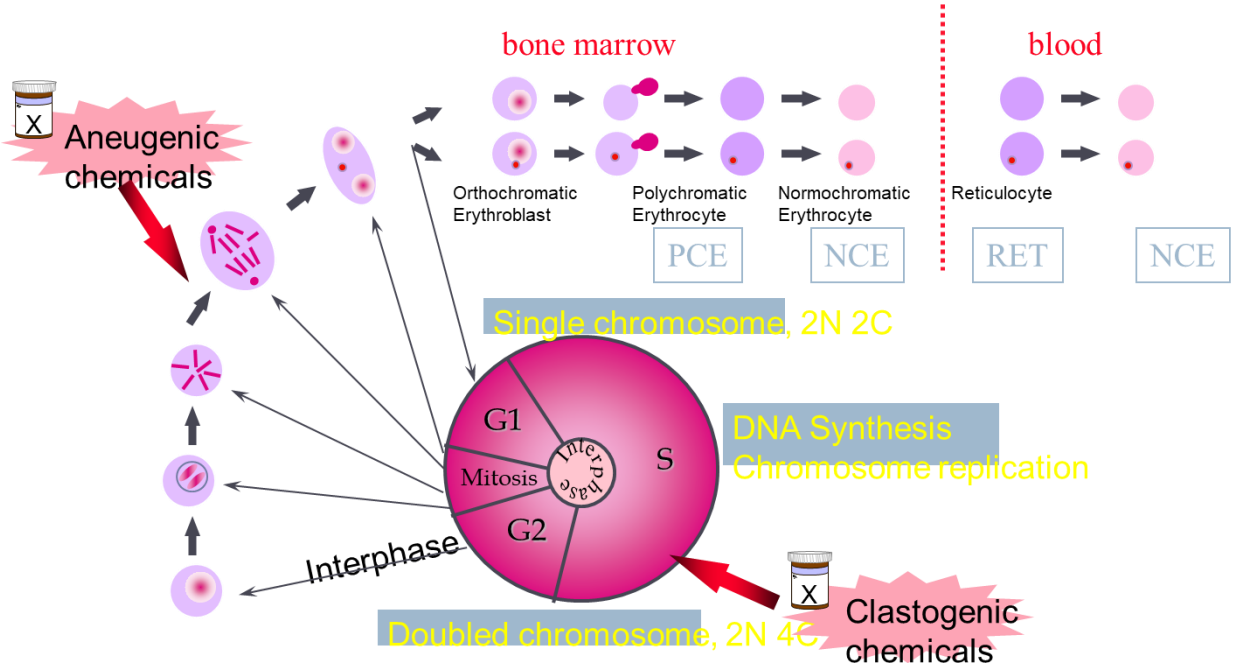
Micronuclei Characteristics:

- micronuclei are morphologically identical to the main nuclei, but are smaller than it.
- it is not linked or connected to the main nuclei.
- it may touch but do not overlap the main nuclei.
- it is non-retractile and they can therefore be readily distinguished from staining particles.



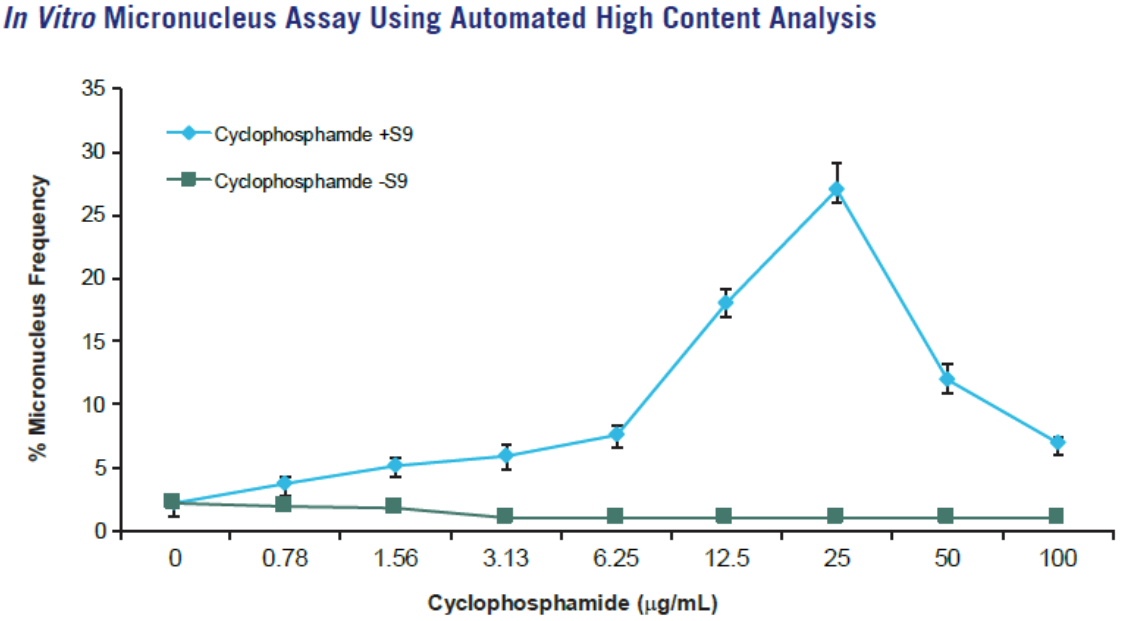
***In vitro* micronucleus test:**

- a recommended regulatory test for genotoxicity that is capable of detecting the micronuclei formation resulting from clastogens or aneugens.
- Clastogens - agents that induce chromosomal breaks mainly through interaction with the DNA
- Aneugens - agents that induce chromosomal loss mainly through interference with the spindle apparatus.
- Cultures are incubated with several concentrations of the test compound for three to four hours in the presence and absence of metabolic activation (S9) and for 21 to 24 hours in the absence of S9.

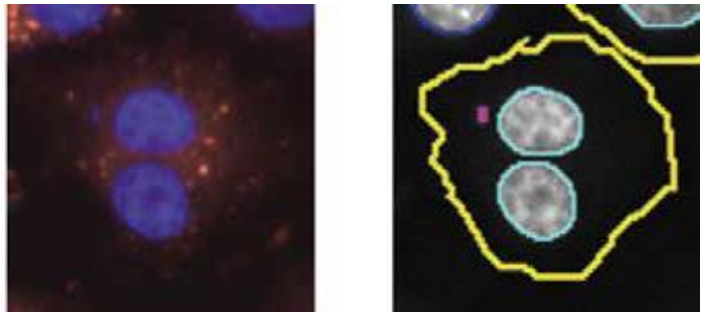


***In vitro* micronucleus test:**

- Micronuclei frequency with cyclophosphamide treatment: Cyclophosphamide, an indirect clastogen, induces an increase of micronuclei frequency only in the presence of metabolic activation (+S9 fraction).



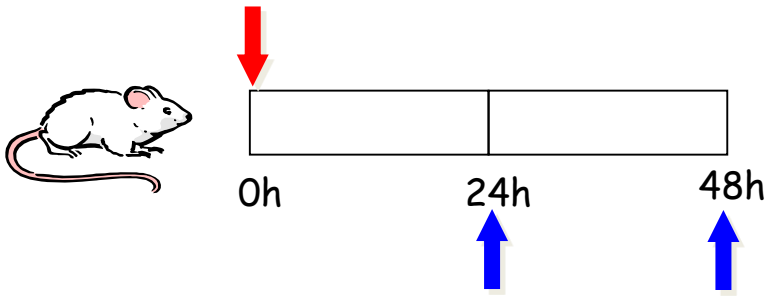
- Example of automated high content analysis of bi-nucleated cells



***In vivo* micronucleus test: design**

- Male and/or female rats or mice are treated with the test compound at three dose levels, usually two or three times at 24-hour intervals.
- Approximately 24 hours after the last dose, bone marrow or peripheral blood is collected to determine the frequency of micronucleated polychromatic erythrocytes (MN-PCEs) or micronucleated reticulocytes (MN-RETs), respectively.
- A positive outcome is characterized by a statistically significant, dose-dependent increase in MN-PCEs or MN-RETs that exceeds historical control limits.

Single dose/Multiple sampling



Species:	mouse/rat/….
Gender:	6 or 7 males in single gender 5 males/5 females
Samples:	bone marrow/peripheral blood