



## Metabolism of xenobiotics

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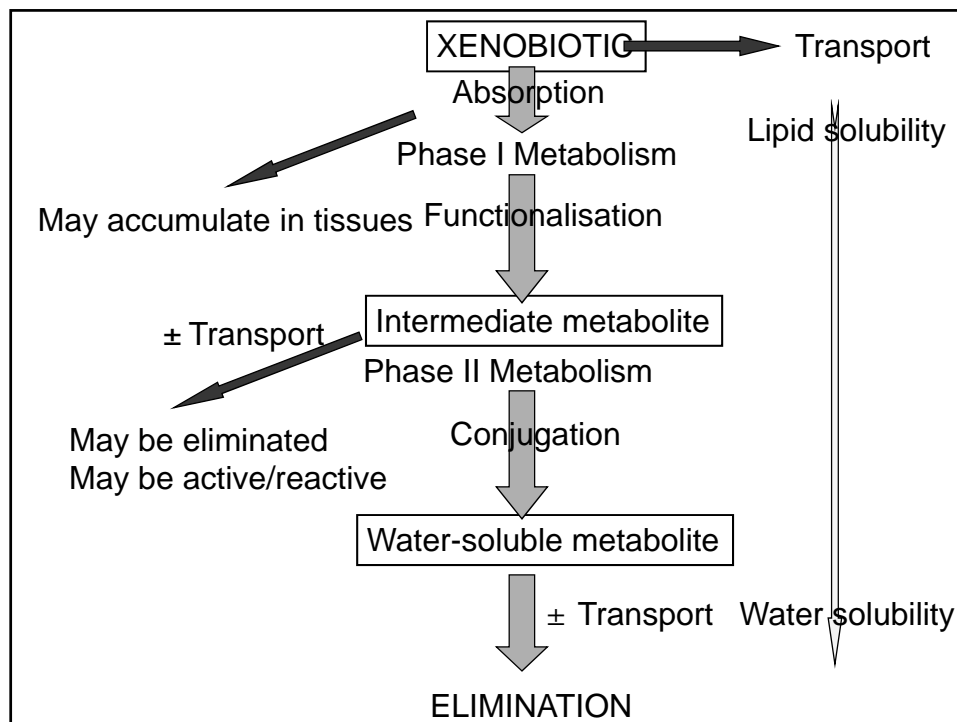
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## Metabolism

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- Xenobiotics are metabolised by enzymes that evolved to eliminate natural compounds
- Metabolism often terminates biological activity
- However, metabolism can also produce, increase or change biological activity
- A single compound may be metabolised by more than one route and by more than one enzyme
- Liver is usually the main site of metabolism, but all tissues have some metabolic capacity



## Phase I metabolism

- Chemical modification (functionalisation) of xenobiotics
- Introduces or uncovers polar functional groups (e.g.  $-\text{OH}$ ,  $\text{NH}_2$ ) that provide sites for Phase II metabolism
- Major classes of reaction:
  - Oxidation
  - Reduction
  - Hydrolysis

## Principal phase I enzymes

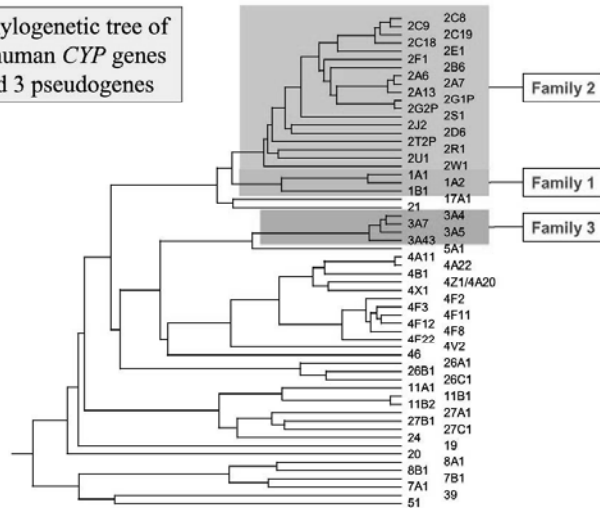
- P450 (CYP)
- Flavin monooxygenase (FMO)
- Monoamine oxidase (MAO)
- Reductases, dehydrogenases, oxidases
- Hydrolases
- Esterases
- Amidases

## P450

- Haemoprotein
- Terminal oxidase of a mixed-function oxidase (MFO) electron-transfer system
- Located in the smooth endoplasmic reticulum of all major organs and tissues
- Uses NADPH as a source of reducing equivalents
- Gene superfamily (human: 57 members in 17 families); families 1-3 (~10 forms) most involved in metabolism of drugs
- Several forms are inducible

## Human CYP super-family

A phylogenetic tree of  
57 human *CYP* genes  
and 3 pseudogenes

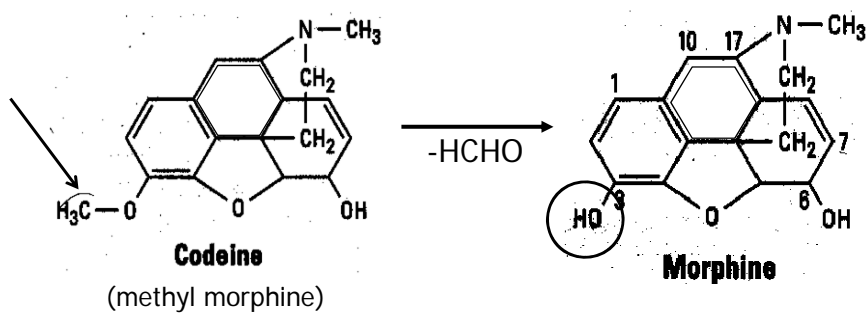


Testa & Kramer (2007)

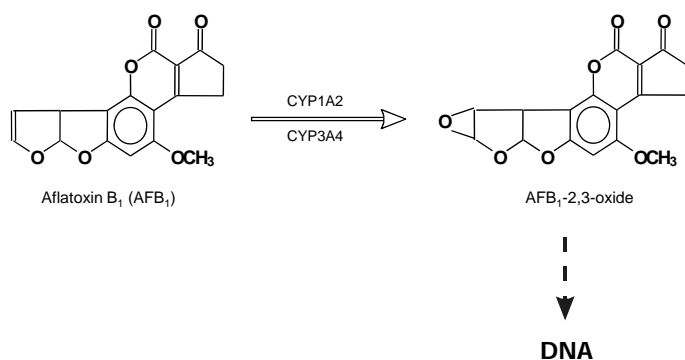
## Reactions catalysed by P450

- Aliphatic hydroxylation
- Aromatic hydroxylation
- Dealkylation
- Deamination
- N-Oxidation
- Sulphoxidation
- Azo- and nitro-reduction

## P450-dependent oxidation



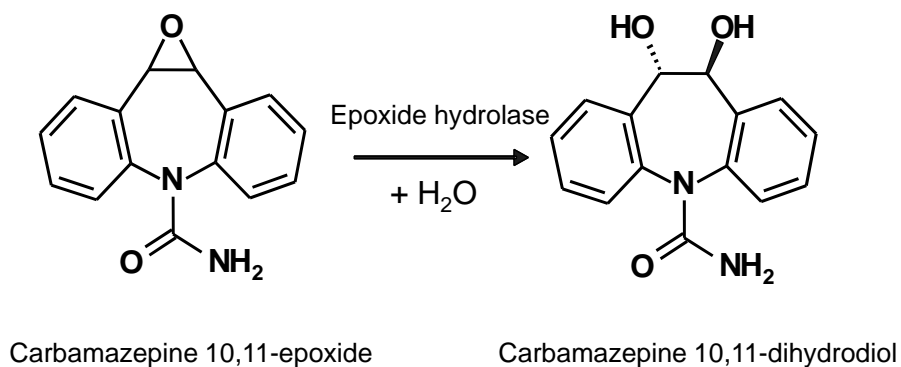
## P450-dependent activation of aflatoxin B<sub>1</sub>



## Epoxide hydrolase

- Two forms
  - EPHX1 – endoplasmic reticulum
  - EPXH2 - cytosol
- Inducible
- Catalyse *trans* addition of water to epoxides

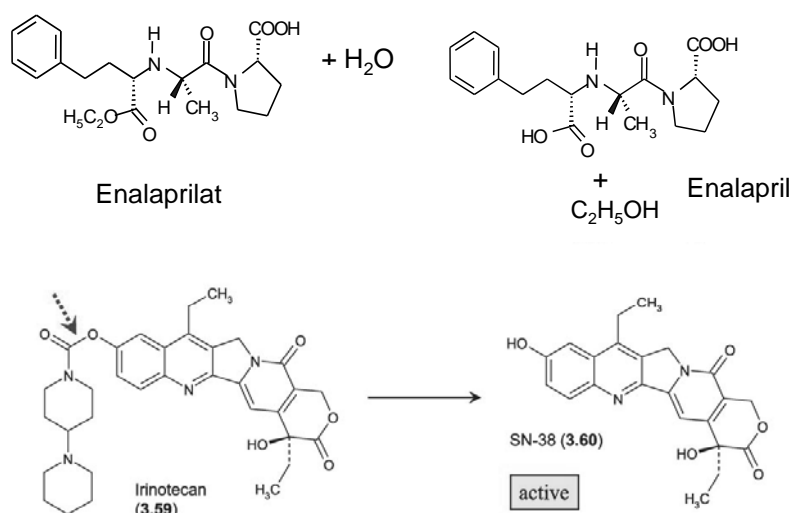
## Epoxide hydrolase reaction



## Esterases

- Carboxylesterases (CESs)
  - Hydrolyse esters to carboxylic acid and alcohol functional groups
  - Several forms: CES1A1, CES2, CES3
- Arylesterases/paraoxonases (PONs)
- Cholinesterases (ChEs)
- Peptidases
- Non-specific esterases in plasma, more substrate-specific forms in liver cytosol

## Carboxylic ester hydrolysis



## Phase II: Conjugation

- Synthetic reaction of a xenobiotic (or of a phase I metabolite of a xenobiotic) with an endogenous substance
- Results in introduction of polar, ionisable group to enhance water solubility and hence excretion

## Important phase II enzymes

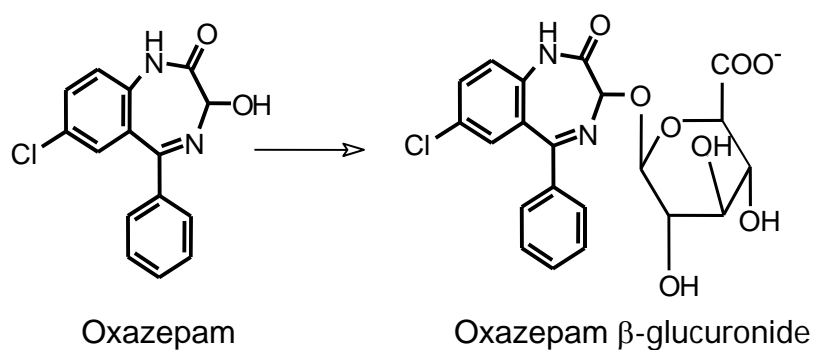
- UDP-Glucuronosyltransferase (UGT)
  - UDP-glucuronic acid (UDPGA)
- Sulphotransferase (SULT)
  - Phosphoadenosyl-phosphosulphate (PAPS)
- Glutathione S-transferase (GST)
  - Glutathione (GSH)
- N-Acetyltransferase (NAT)
  - Acetyl coenzyme A (AcetylCoA)



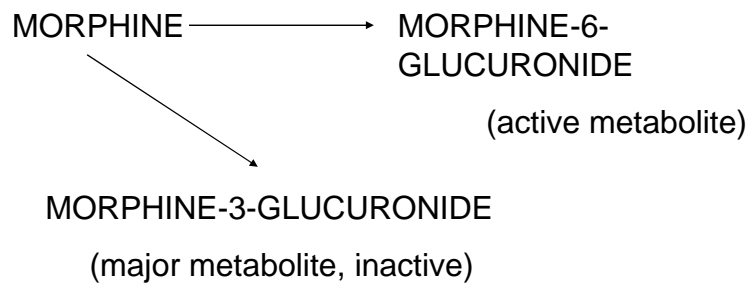
## Human UDP glucuronosyltransferases (UGTs)

- Conjugating moiety: glucuronic acid, a sugar
- Co-factor: UDP- $\alpha$ -D-glucuronic acid (UDPGA), derived from glycogen synthesis
- Located in endoplasmic reticulum
- 4 families of UGTs (22 forms)
  - UGT1A1...10, UGT2A1...3, UGT2B4...28, UGT3A1-2, UGT8A
- Some forms inducible
- Substrates: -OH, amino, thiol, (carbon)

## Glucuronidation reaction



## Morphine metabolism

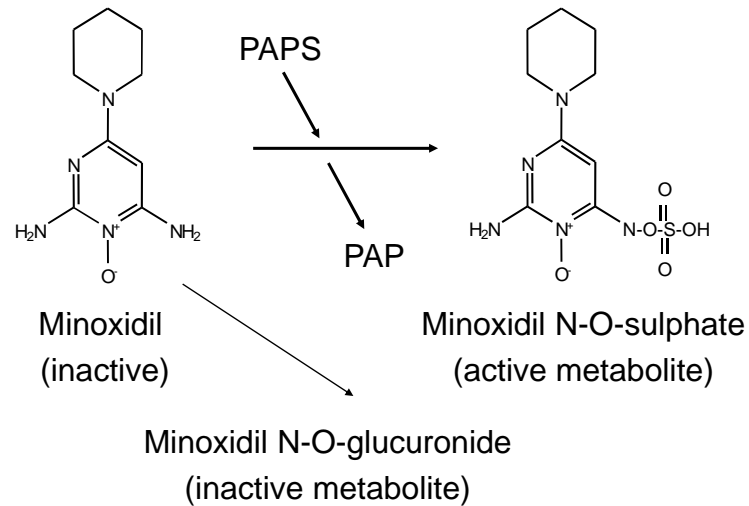


Morphine also undergoes N-demethylation

## Human sulphotransferases (SULTs)

- Produces sulphate conjugates
- Soluble enzymes
- Produce ethereal sulphates (R-O-SO<sub>3</sub>H) from –OH and sulphamates (from –NH<sub>2</sub>)
- Noninducible
- Utilise 3'-phosphoadenosine-5'-phosphosulphate (PAPS) as the sulphate donor
- 4 Families of SULTs (13 forms)
  - SULT1A, 1B, 1C, 1E; SULT2A, 2B; SULT4A; SULT6B

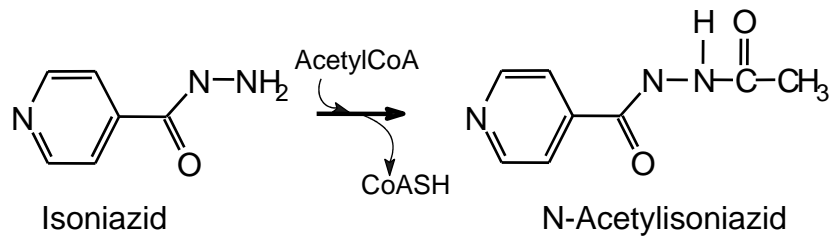
## Sulphation reaction



## N-Acetylation

- N-Acetyltransferases (NAT)
- Substrates: aromatic amines, sulphonamides
- Conjugating moiety: acetyl group
- Co-factor: acetyl-CoA
- Few forms: NAT1, NAT2

## Acetylation reaction



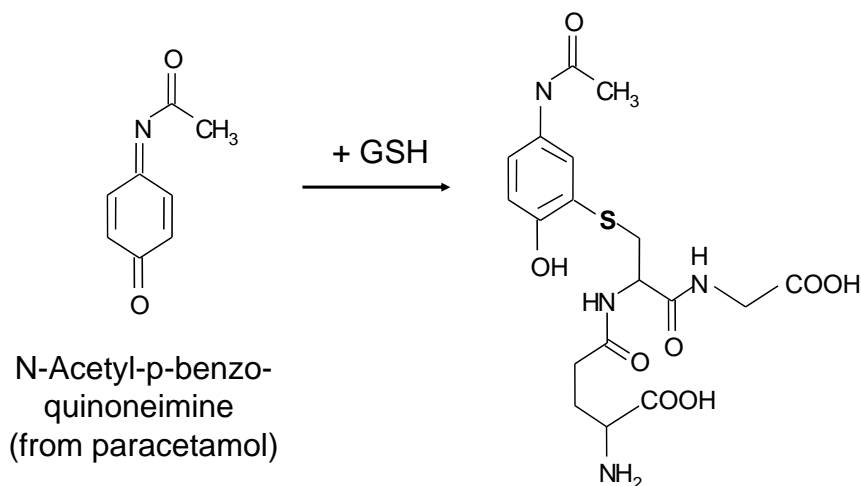
### Other substrates:

Hydralazine  
Phenylzine  
Dapsone

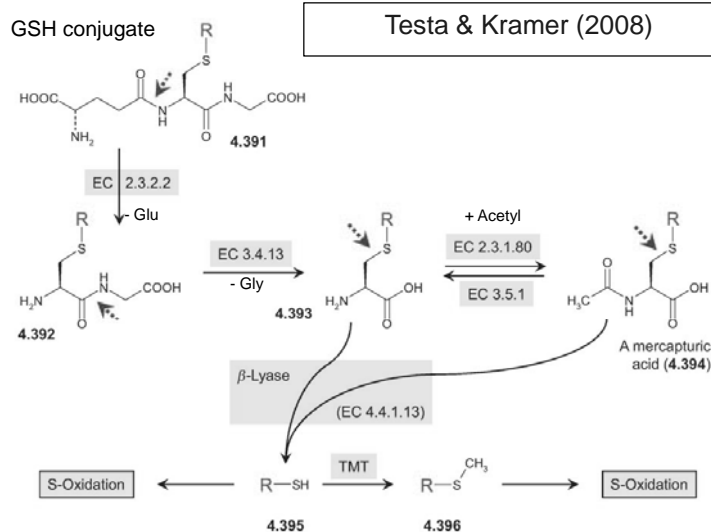
## Conjugation with glutathione

- Glutathione S-transferases (GST)
- Substrates: epoxides, electrophiles, organic halides
- Conjugating moiety: glutathione
- Co-factor: None
- Mainly in cytosol
- Inducible
- 4 families of soluble GSTs (13 forms)
  - GSTA (alpha), GSTM (mu), GSTP (pi), GSTT (theta)

## Glutathione conjugation reaction



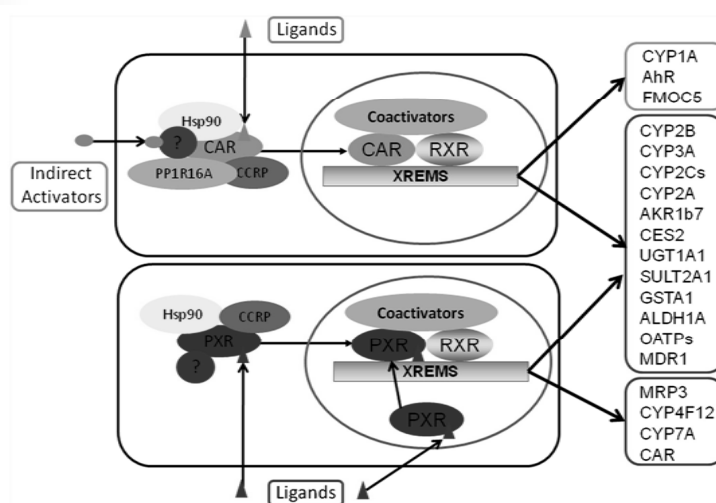
## Mercapturic acid pathway



## Other phase II reactions

- Glycine conjugation
- Other amino acid conjugations
- Methylation
- Transulphuration

## Activation of CAR and PXR



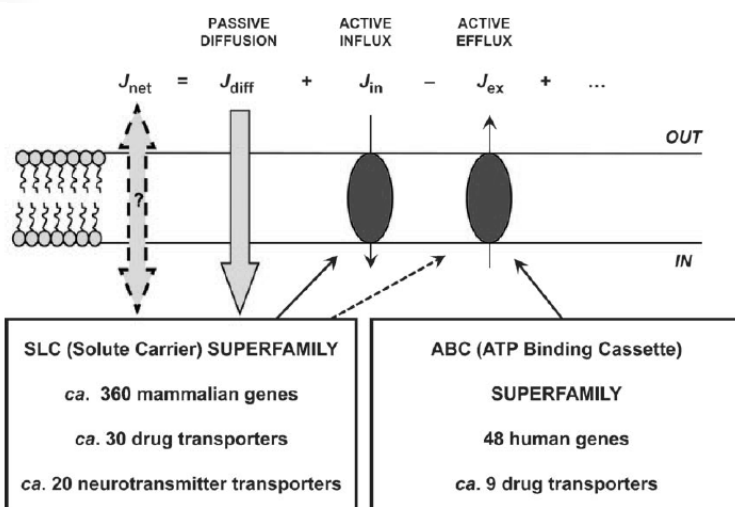
Tolson & Wang (2010)

## Drug metabolism

Metabolism:

- Serves to eliminate drugs
- Usually produces more hydrophilic compounds
- Usually produces less active (less toxic) products (metabolites)
- Exhibits large interindividual variation
- Can be influenced by environmental factors (e.g. diet and smoking)
- Is cause of many drug-drug, drug-diet, and drug-herbal interactions
- Extent may be magnified by enterohepatic recycling and “double jeopardy”

## The two super-families of drug transporters

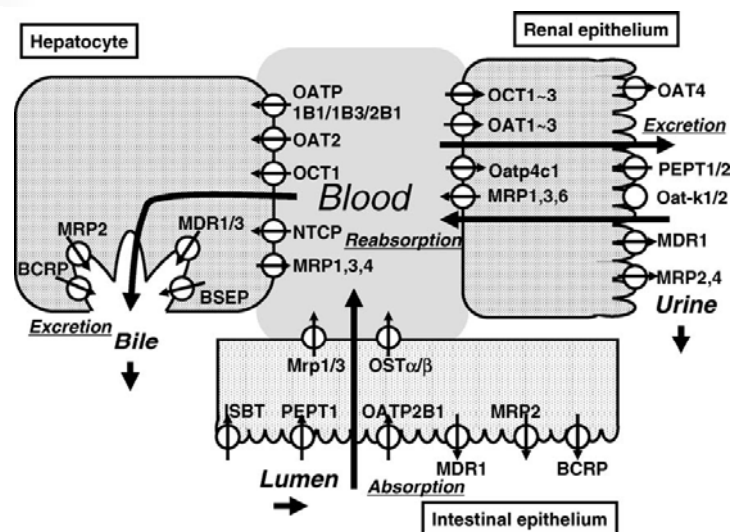


Scherrmann (2009)

## Major drug transporters

- ATP-Binding Cassette Transporters (ABC)
  - P-glycoprotein (P-gp or MDR1)
  - Multidrug Resistance Associated Proteins (Mrps)
  - Breast cancer resistant protein (BCRP)
- Solute Carriers (SLC)
  - Organic anion transporting polypeptides (OATPs)
  - Organic anion transporters (OATs)
  - Organic cation transporters (OCTs)
  - Nucleoside transporters (ENTs, CNTs)
  - Oligopeptide transporters (PepTs)
  - Bile acid transporters (e.g. NTCPs)
  - Monocarboxylate transporters (MCTs)

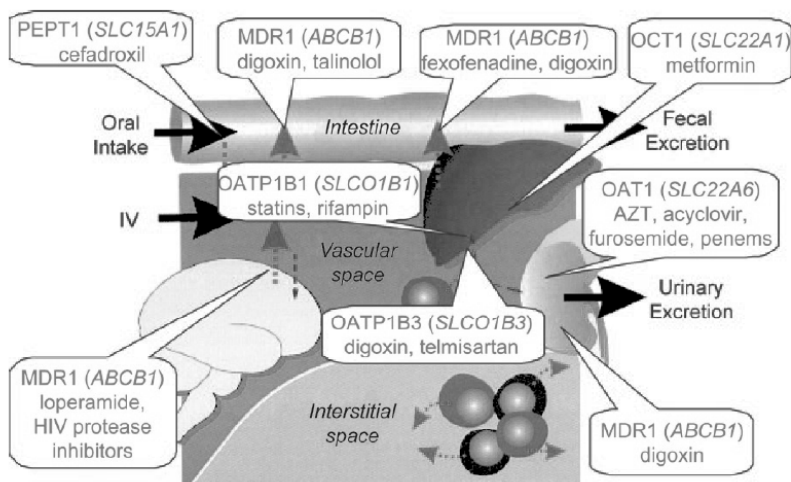
## Drug transporters



Shitara et al 2006

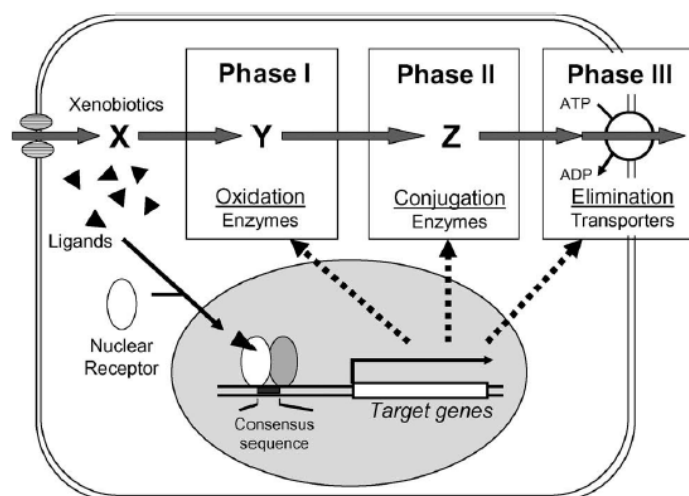


### Some drugs, the pharmacokinetics of which are influenced by transporters



Kohl (2009)

### Phases of drug disposition



Nakata et al (2006)

## Characteristics of xenobiotic metabolising enzymes and transporters

- Often multi-gene families
- Unique but overlapping specificities
- Marked tissue and often sex (esp. rodents) differences in expression
- Important quantitative and qualitative differences amongst species
- Subject to genetic, environmental and pathophysiological variability

## References

- Relevant chapters in
  - Principles of Biochemical Toxicology (Timbrell), Taylor & Francis
  - Casarett and Doull's Toxicology, McGraw Hill
- Shitara *et al* (2006). *Eur J Pharm Sci.* **27**:425-446
- Guengerich (2006). *AAPS J.* **8**: E101-111
- Testa (2009). *Chem Biodivers.* **6**:2055-2070