Role of Metabolism

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Philippus Theophrastus
Aureolus
Bombastus von Hohenheim
PARACELSUS
(1493 - 1541)

“The dose makes the poison.”

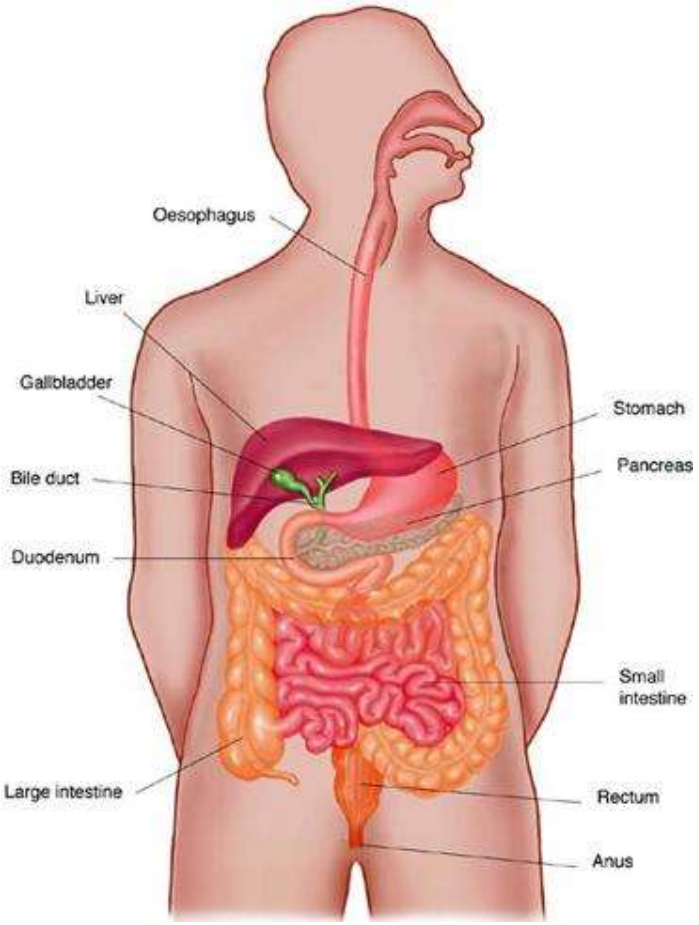
Structure of the presentation

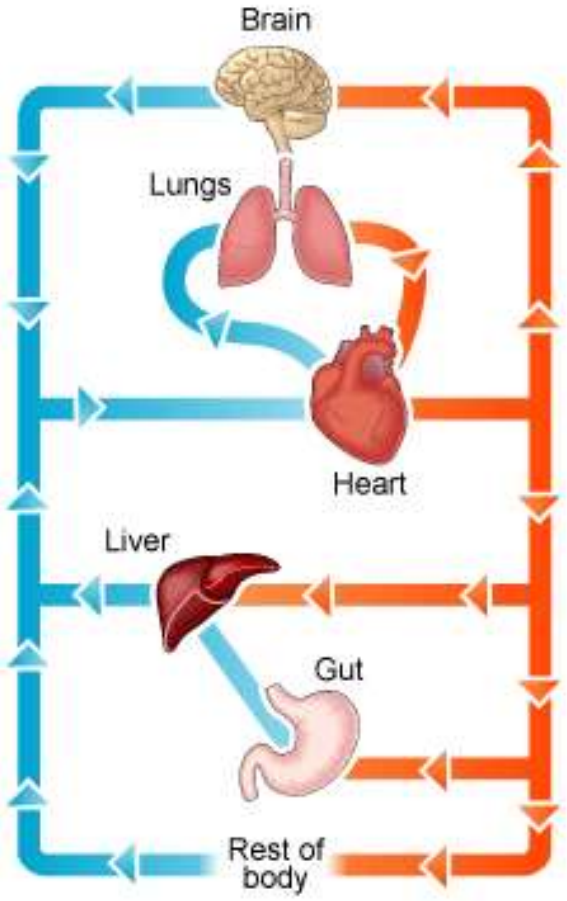
- Basic principles

- Role of metabolism - examples

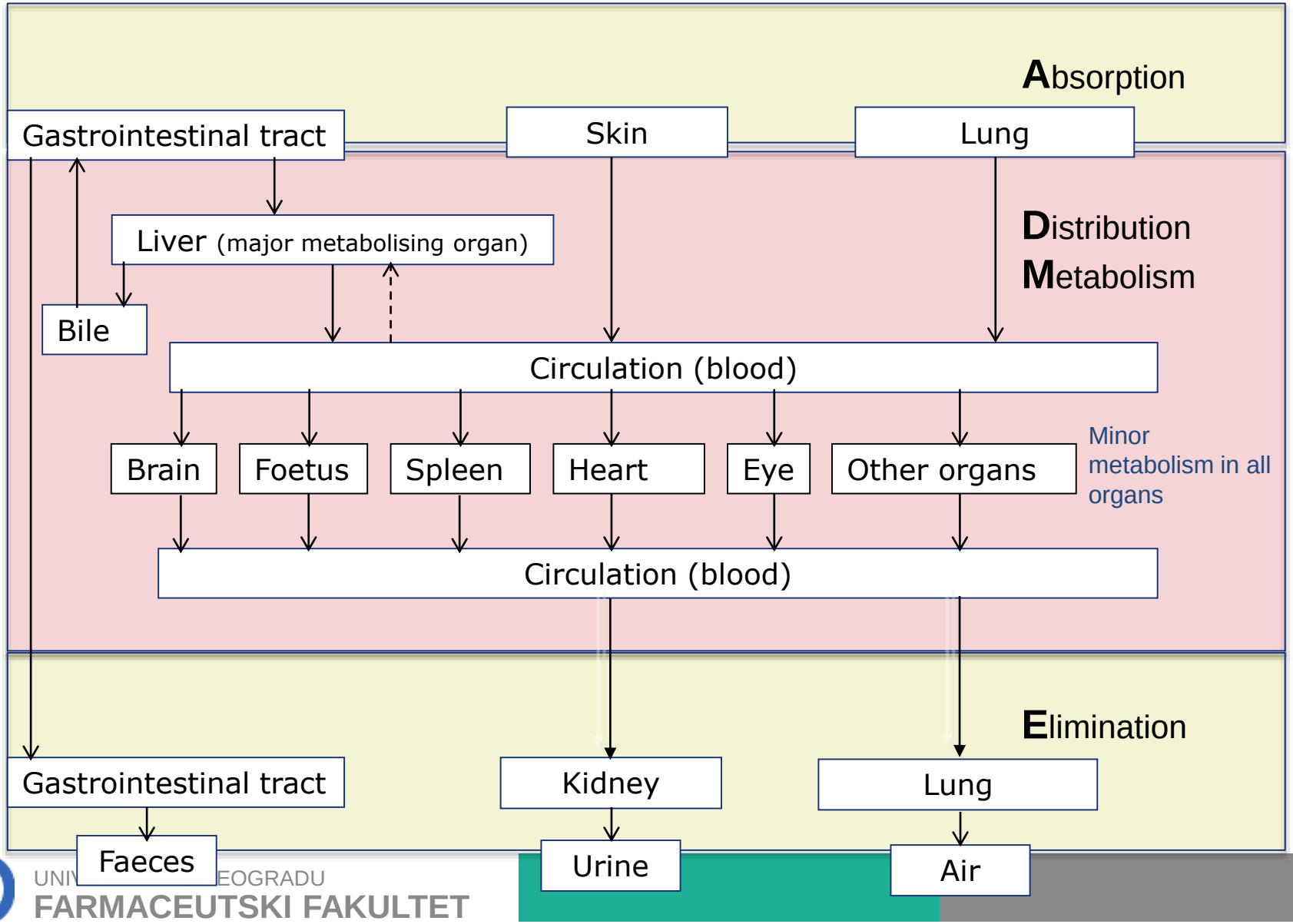
A chemical's way through the human body

The acronym ADME stands for **a**bsorption, **d**istribution, **m**etabolism and **e**limination.





ADME and Toxicokinetics



What is metabolism?

Basic rule

Poison biotransformation or metabolism is the process of converting lipophilic (fat soluble) chemicals, which are readily absorbed from the gastrointestinal tract and other sites, into hydrophilic (water soluble) chemicals, which are readily excreted in urine or bile.

Metabolism and its relevance

- Mechanistic toxicology

  Understanding the mechanism of toxicity

- Clinical toxicology

  Therapy optimization, monitoring of exposure

- Analytical toxicology

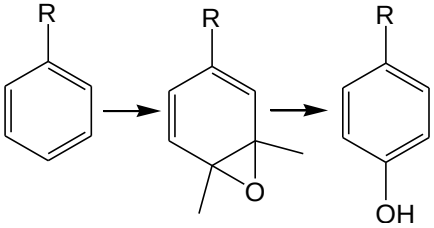
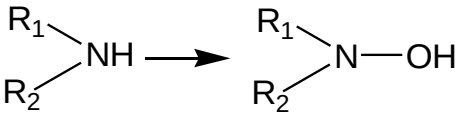
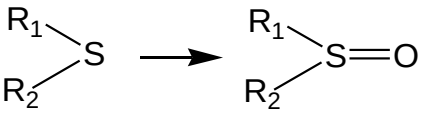
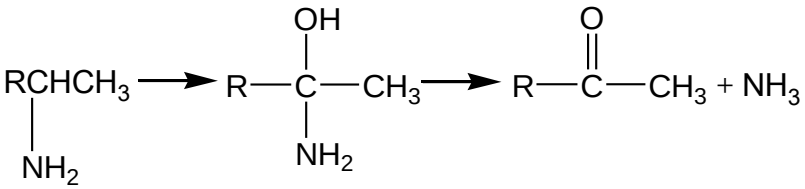
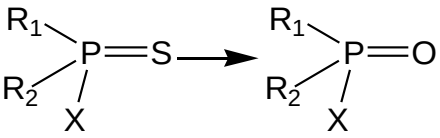
  Methodology optimization *vs* relevant metabolite

Reactions involved in metabolism

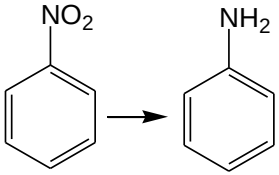
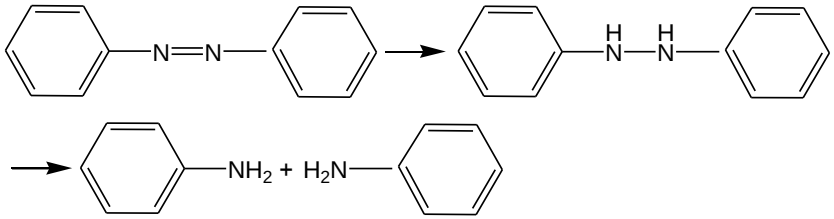
- Phase I metabolism (metabolic transformation)
  - Oxidation
  - Reduction
  - Hydrolysis

- Phase II metabolism (conjugation)
  - Glucuronidation
  - Glutathione conjugation
  - Sulfatation
  - Acetylation
  - Methylation
  - Amino acid conjugation

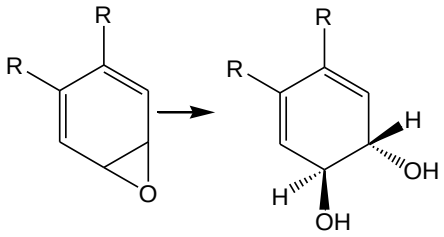
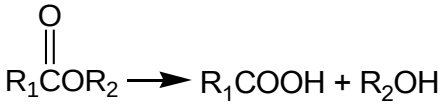
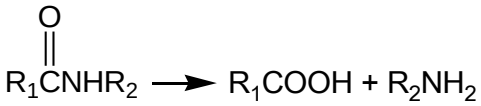
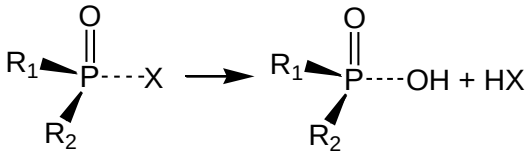
Major reactions involved in metabolism

	Reactions	Examples
Oxidation		
Alyphatic hydroxylation	$RCH_2CH_3 \rightarrow RCHOHCH_3$	Ethyl-, methyl alcohol
Aromatic hydroxylation		Benzene, toluene, xylene
N-oxidation	$RNH_2 \rightarrow RNHOH$ 	Aniline, chlorpheniramine
S-oxidation		Chlorpromazine
N-dealkylation	$RNHCH_3 \rightarrow RNH_2 + CH_2O$	Imipramine, diazepam
O-dealkylation	$ROCH_3 \rightarrow RNH_2 + CH_2O$	Codeine
Deamination		Benzodiazepines
Desulfuration		Organophosphates

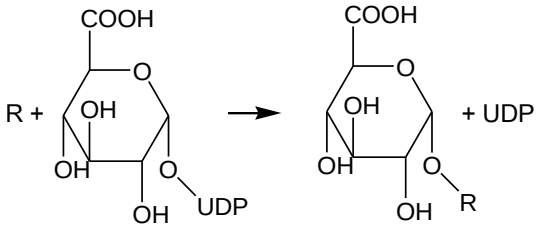
Major reactions involved in metabolism

	Reactions	Examples
Reduction		
Ketones/aldehydes	$R_1R_2CO \rightarrow R_1R_2CHOH$	Haloperidol
Aromatic nitro		Nitrobenzene
Azo reduction		o-Aminoazotoluene
As reduction	$As^{5+} \rightarrow As^{3+}$	Tryparsamide
Disulfide reduction	$R_1R_2NCSS\text{-}SSCNR1R_2 \rightarrow 2R_1R_2NCSSH$	Disulfiram

Major reactions involved in metabolism

Reactions	Examples
Hydrolysis	
	Carbamazepine
	Aspirin, cocaine
	Procainamide
	Organophosphates

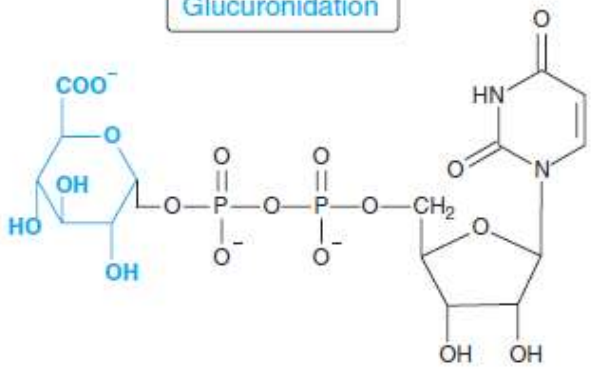
Major reactions involved in metabolism

	Reactions	Examples
Conjugation		
Glucuronidation	 UDP-glucuronic acis	Morphine, benzene, diazepam
Sulfation	PAPS + ROH → R-O-SO_2-OH + PAP 3'-phosphoadenosine--5'-phosphosulphate → 3'phosphoadenosine-5'--phosphate	Benzene, Toluene, Acetaminophen
Acetylation	CoAS-CO-CH_3 + RNH_2 → RNH-CO-CH_3 + CoA-SH	Sulfonamides
Methylation	RO-, RS-, RN- + AdoMet → RO-CH_3 + AdoHomCys S-adenosylmethionine	Captopril
Glutathione	GSH + RX → GS-R + HX	Acetaminophen
Amino acids	Gly + RCOOH → RCOO-$NHCH_2COOH$ Gly – R-NH_2 → R-NH-$OCOCH_2NH_2$	Toluene, Xylene

Phase II – Conjugation (1)

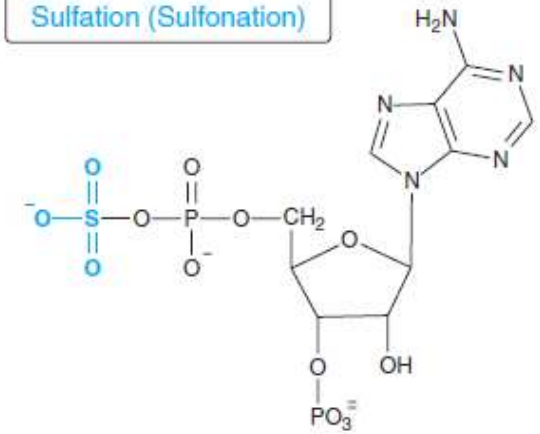
Structures of cofactors for conjugation reactions

Glucuronidation



Uridine-5´-diphospho-α-D-glucuronic acid (UDP-GA)

Sulfation (Sulfonation)

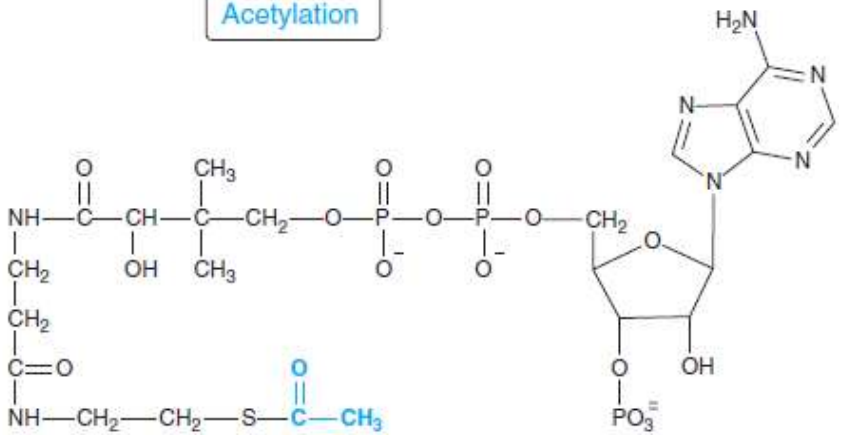


3´-Phosphoadenosine-5´-phosphosulfate (PAPS)

Phase II – Conjugation (2)

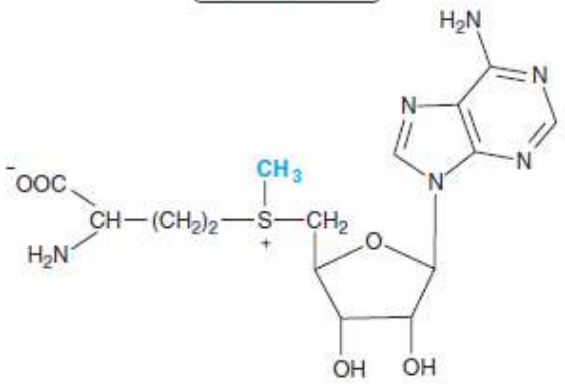
Structures of cofactors for conjugation reactions

Acetylation



Acetyl coenzyme A

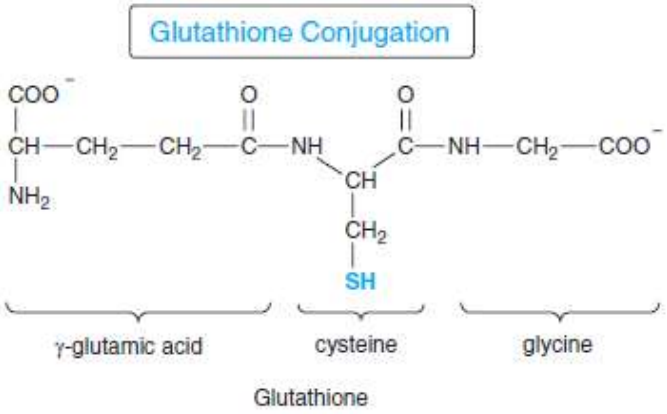
Methylation

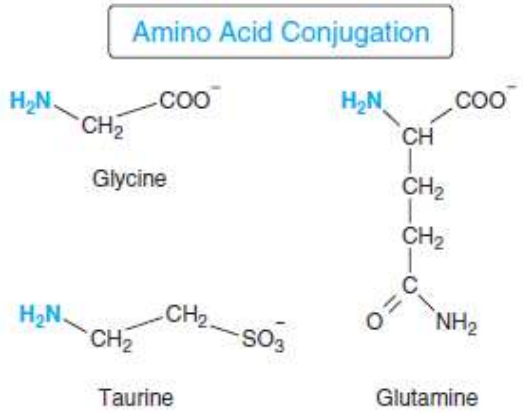


S-Adenosylmethionine (SAM)

Phase II – Conjugation (3)

Structures of cofactors for conjugation reactions

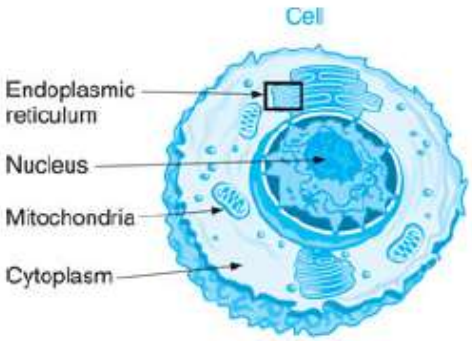


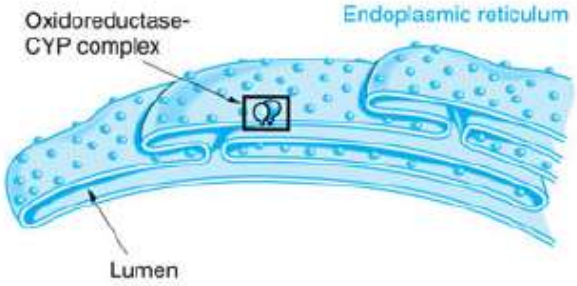


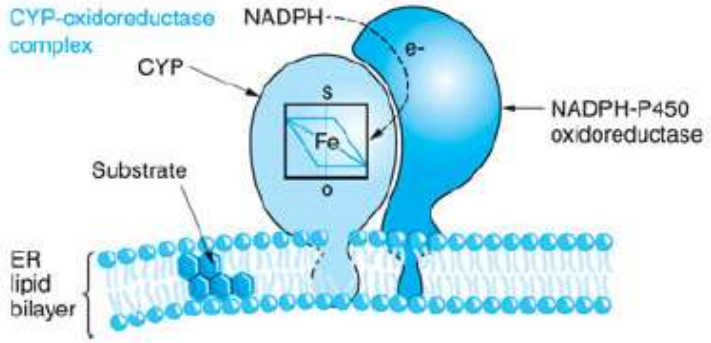
Enzymes involved in metabolism

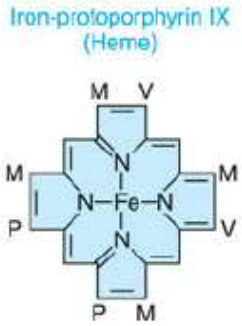
REACTION	ENZYME OR SPECIFIC REACTION	LOCALIZATION
Oxydation	Cytochrome P450	Microsomes
	Flavin-monooxygenases	Microsomes
	Monoamine oxidase	Mitochondria
	Peroxidase	Microsomes, lysosomes, saliva
	Diamine oxidase	Cytosol
	Xanthine oxidase	Cytosol
	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogense	Mitochondria, cytosol
	Aldehyde oxidase	Cytosol
Reduction	Azo-, nitro-reduction	Microsomes
	Carbonyl (aldo/keto) reduction	Microsomes, cytosol
	Disulfide reduction	Cytosol
	Sulfoxide reduction	Cytosol
	Quinone reduction	Microsomes, cytosol
	Reductive dehalogenation	Microsomes
	Dehydroxilation	Cytosol
Hydrolysis	Carboxylesterase	Microsomes, cytosol, lysosomes, blood
	Phosphorylphosphatese	Cytosol, lysosomes, blood
	Epoxide hydrolaze	Microsomes, cytosol
Conjugation	UDP-Glucuronosyltransferase	Microsomes
	Sulfotransferase	Cytosol
	Glutathione transferase	Cytosol, microsomes, mitochondria
	Amino acid transferase	Microsomes, mitochondria
	N-Acetyltransferase	Mitochondria, cytosol
	Methyltransferase	Cytosol, microsomes, blood

Enzymes involved in oxidation (1): CYPs









Cytochrome P450s (P450 or CYP)
1. Hydroxylation of an aliphatic or aromatic carbon;
2. Epoxidation of a double bond;
3. Heteroatom oxygenation and N-hydroxylation;
4. Heteroatom (O-, S-, and N-) dealkylation;
5. Oxidative group transfer;
6. Cleavage of esters;
7. Dehydrogenation.

CYPs are embedded in the phospholipid bilayer of the endoplasmic reticulum (ER). Most of the enzyme is located on the cytoplasmic surface of the ER. A second enzyme, NADPH-cytochrome P450 oxidoreductase, transfers electrons to the CYP where it can, in the presence of O2, oxidize xenobiotic substrates, many of which are hydrophobic and dissolved in the ER. A single NADPH-CYP oxidoreductase species transfers electrons to all CYP isoforms in the ER. Each CYP contains a molecule of iron-protoporphyrin IX that functions to bind and activate O2. Substituents on the porphyrin ring are methyl (M), propionyl (P), and vinyl (V) groups.

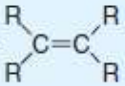
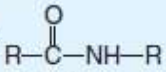
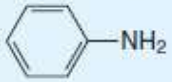
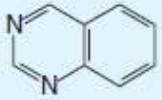
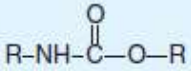
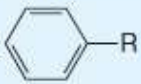
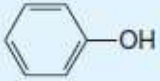
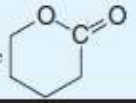
Enzymes involved in oxidation (2)

FLAVIN-CONTAINING MONOOXYGENASES (FMOs) are superfamily of phase 1 enzymes that are expressed at high levels in the liver and localized to the ER. FMOs oxidize the nucleophilic nitrogen, sulfur, and phosphorus heteroatom of a variety of xenobiotics. FMOs are minor contributors to drug metabolism and generally produce benign metabolites. FMOs are not induced by any of the xenobiotic receptors or easily inhibited (in distinction to CYPs).

MONOAMINE OXIDASE (MAO) is involved in the oxidative deamination of primary, secondary, and tertiary amines.

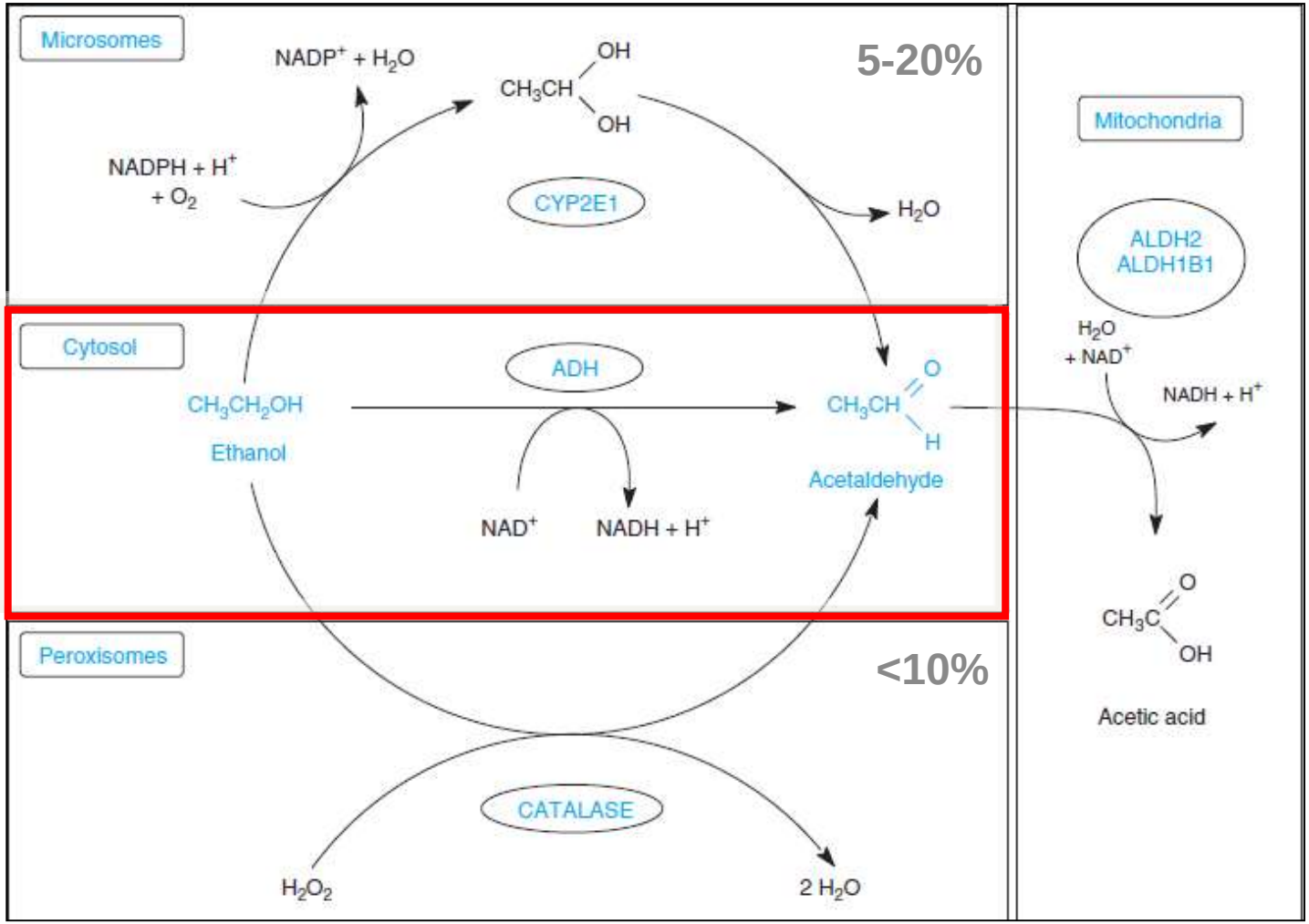
PEROXIDASE are heme-containing enzymes that couple the reduction of hydrogen peroxide (or a lipid hydroperoxide) to the oxidation of other substrates. However, peroxidases do play an important role in xenobiotic toxicity, especially the activation of xenobiotics (including the activation of proximate carcinogens to ultimate carcinogens) in skin, bladder, bone marrow, and various other extrahepatic tissues (aflatoxin).

Chemical Groups and Enzymes Possibly Involved in Their Metabolism

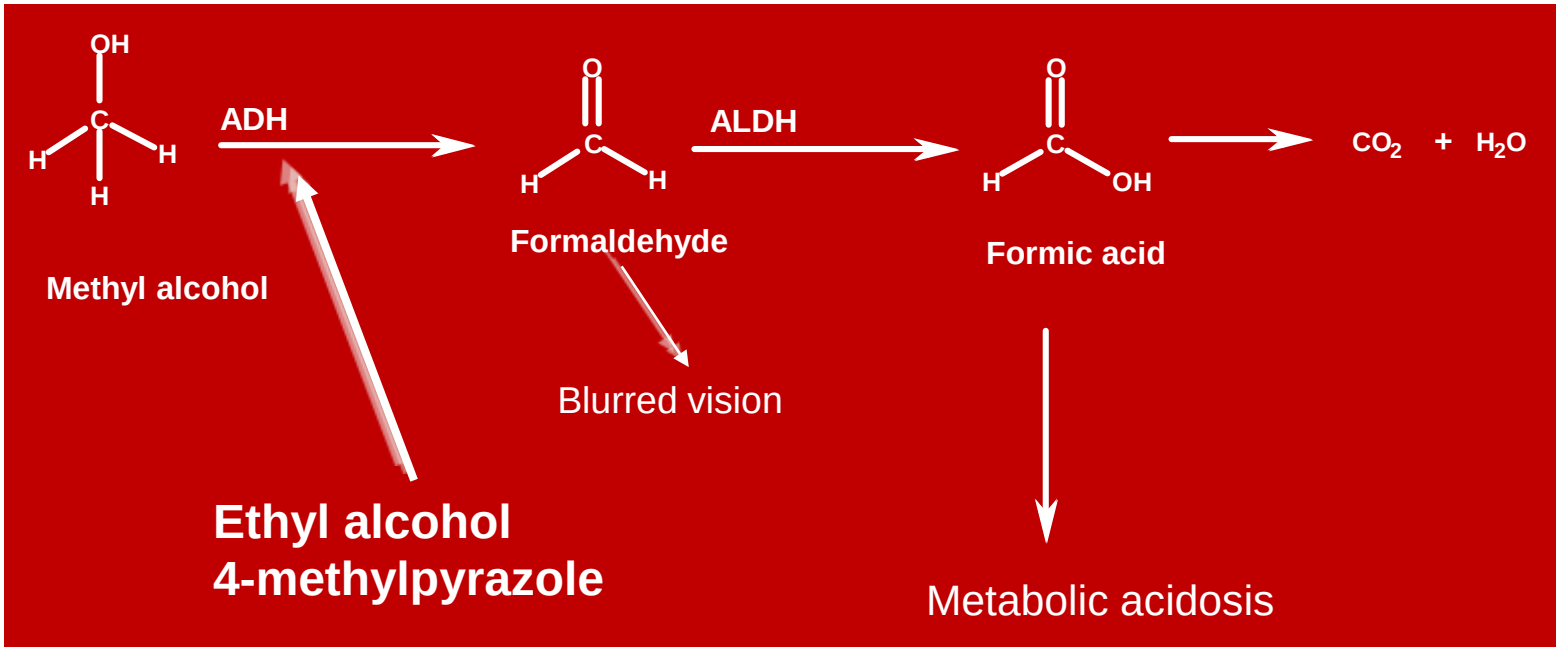
CHEMICAL GROUP	ENZYME(S)	REACTION(S)	CHEMICAL GROUP	ENZYME(S)	REACTION(S)
Alkane $R-CH_2-R$	CYP	Hydroxylation, dehydrogenation	Aldehyde 	CYP, ALDH	Oxidative de-formylation, oxidation to carboxylic acid
Alkene 	CYP, GST	Epoxidation, glutathione adduct formation	Amide 	Amidase (esterase)	Hydrolysis
Alkyne $R-C\equiv C-R$	CYP	Oxidation to carboxylic acid	Aniline 	CYP, NAT, UGT, peroxidase, SULT	N-Hydroxylation, N-acetylation, N-glucuronidation, N-oxidation, N-sulfonation
Aliphatic alcohol $R-CH_2-OH$	CYP, ADH, catalase, UGT, SULT	Oxidation, glucuronidation, sulfonation	Aromatic azaheterocycles 	UGT, CYP, aldehyde oxidase	N-Glucuronidation, hydroxylation, N-oxidation, ring cleavage, oxidation
Aliphatic amine $R-NH_2$	CYP, FMO, MAO, UGT, SULT, MT, NAT, peroxidase	N-Dealkylation, N-oxidation, deamination, N-glucuronidation, N-carbamoyl glucuronidation, N-sulfonation, N-methylation, N-acetylation	Carbamate 	CYP, esterase	Oxidative cleavage, hydrolysis
Amidine $HN=CR-NH_2$	CYP	N-Oxidation	Ester $R-CH_2-O-C(=O)-R$	CYP, esterase	Oxidative cleavage, hydrolysis
Arene 	CYP	Hydroxylation and epoxidation	Ether $R-CH_2-O-CH_2-R$	CYP	O-Dealkylation
Carboxylic acid $R-COOH$	UGT, amino acid transferases	Glucuronidation, amino acylation	Ketone $R-CH_2-C(=O)-R$	CYP, SDR, AKR	Baeyer–Villiger oxidation, reduction
Epoxide 	Epoxide hydrolase, GST	Hydrolysis, glutathione adduct formation	Phenol 	CYP, UGT, SULT, MT	Ipso-substitution, glucuronidation, sulfonation, methylation
Lactone 	Lactonase (paraoxonase)	Hydrolysis (ring opening)	Thioether $R-CH_2-S-CH_2-R$	CYP, FMO	S-Dealkylation, S-oxidation

ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; AKR, aldo-keto reductases; FMO, flavin monoxygenase; GST, glutathione transferase; MAO, monoamine oxidase; MT, methyltransferase; SDR, short-chain dehydrogenases/reductases; NAT, N-acetyltransferase; SULT, sulfotransferase; UGT, UDP-glucuronosyltransferase.

Oxidation of ethyl alcohol to acetic acid by cytochrome P450 (CYP2E1), **alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH)** and catalase

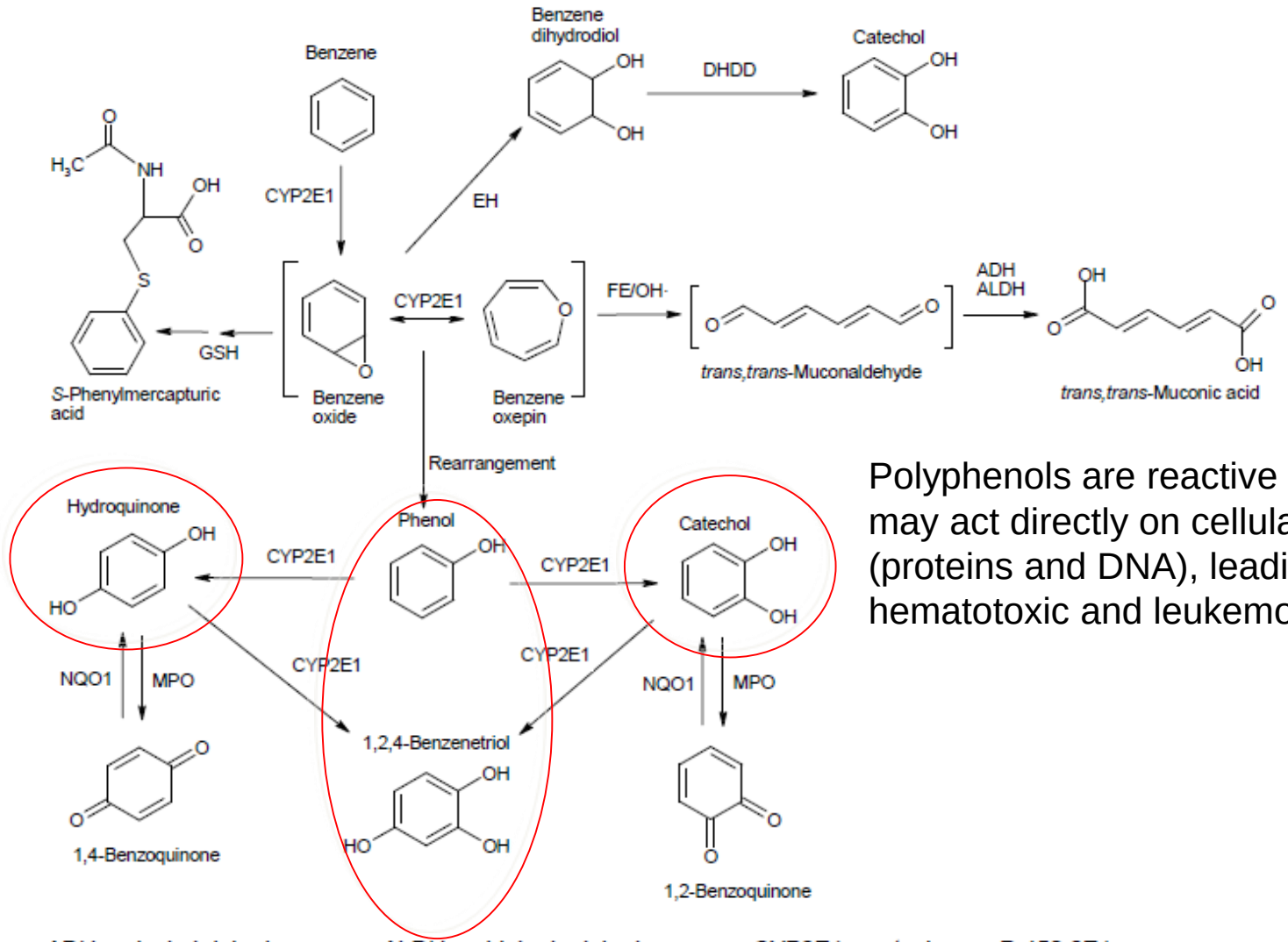


Metabolism *vs.* antidotal treatment



Methyl and ethyl alcohols share the same metabolic pathway.
4-methylpyrazole inhibits enzyme ADH.
Ethyl alcohol or 4-methylpyrazole are used in acute methyl alcohol poisoning.

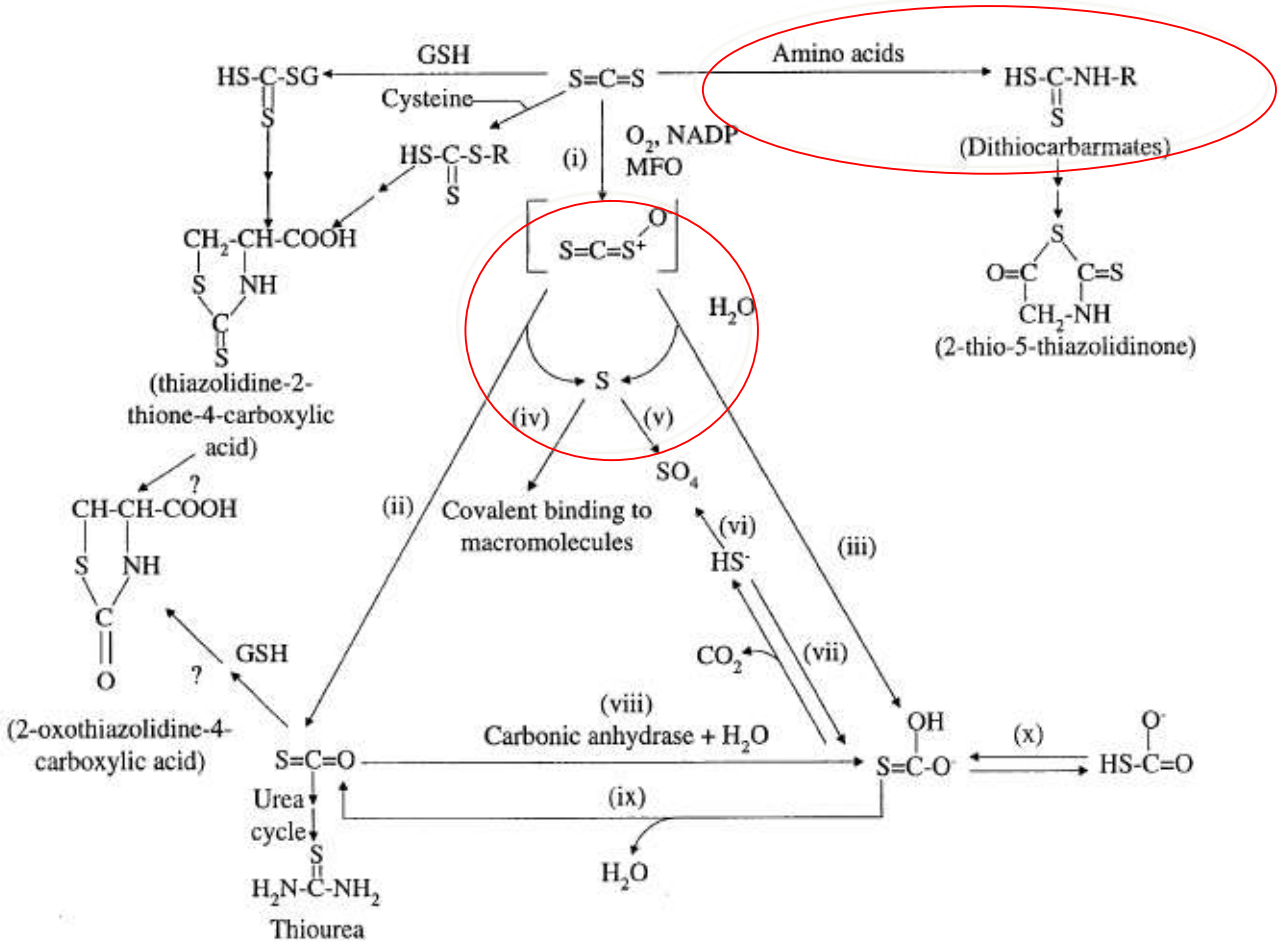
Metabolism of benzene *vs.* its mechanism of toxicity



Polyphenols are reactive products that may may act directly on cellular macromolecules (proteins and DNA), leading to hematotoxic and leukemogenic effects.

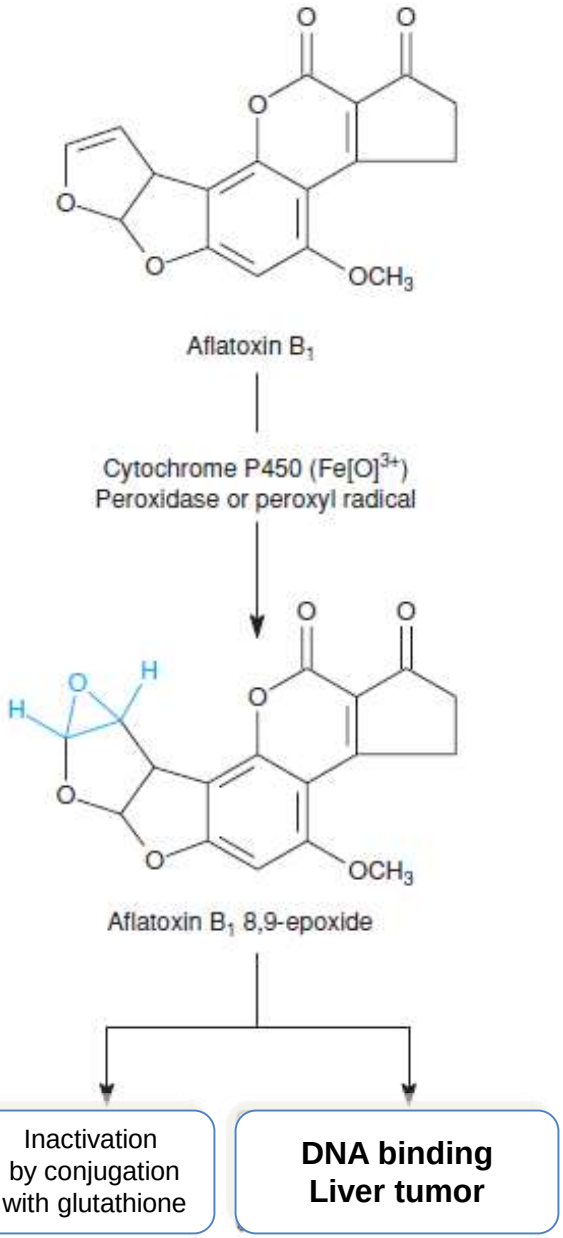
ADH = alcohol dehydrogenase; ALDH = aldehyde dehydrogenase; CYP2E1 = cytochrome P-450 2E1;
DHDD = dihydrodiol dehydrogenase; EH = epoxide hydrolase; GSH = glutathione; MPO = myeloperoxidase;
NQ01 = NAD(P)H:quinone oxidoreductase

Metabolism of carbon disulfide *vs.* its mechanism of toxicity



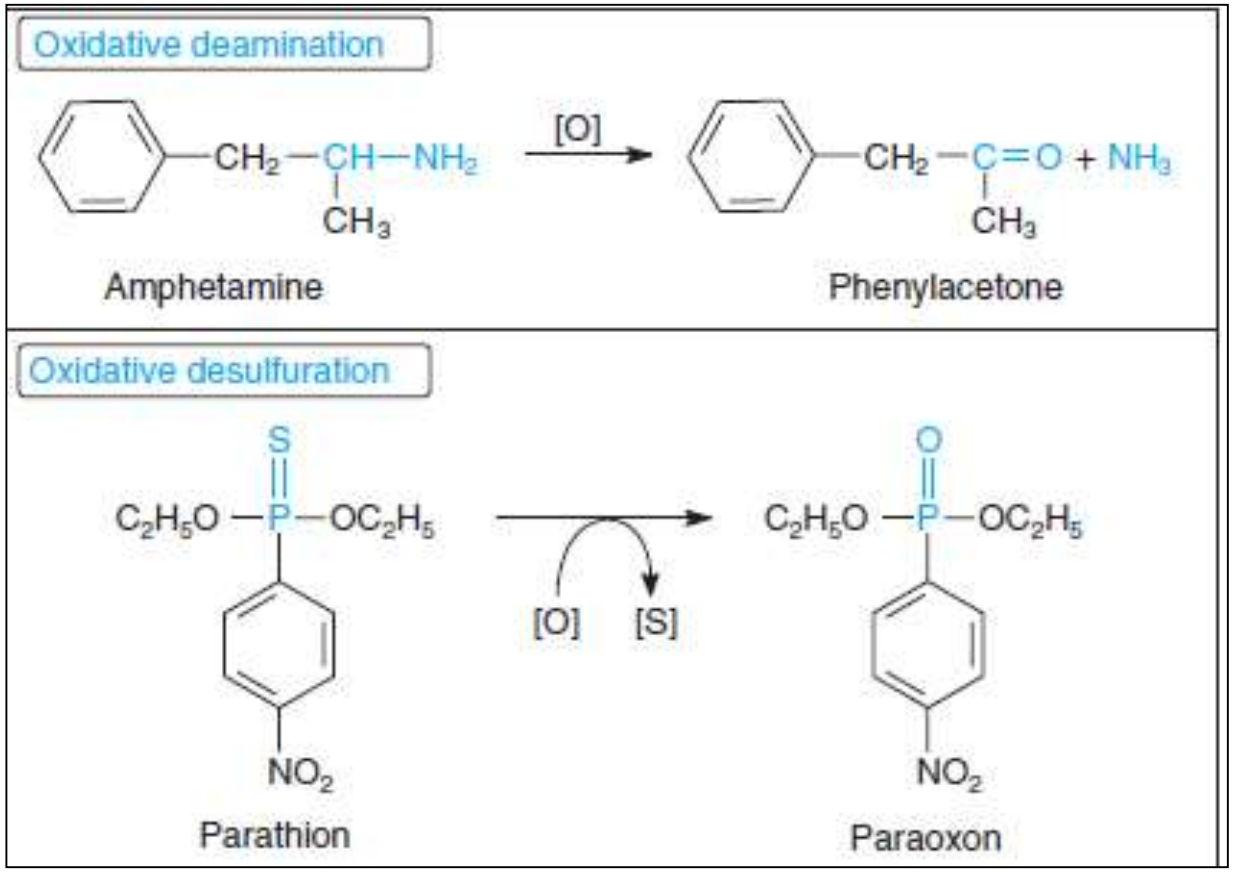
Formation of dithiocarbamates may in part account for peripheral neurotoxicity.

Metabolism *vs.* mechanism of toxicity

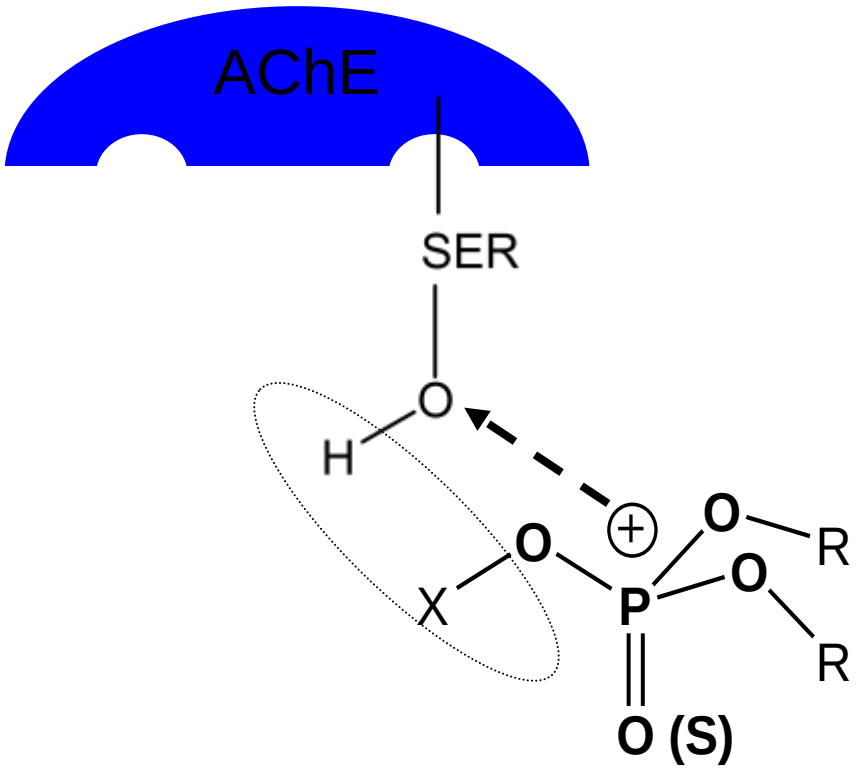


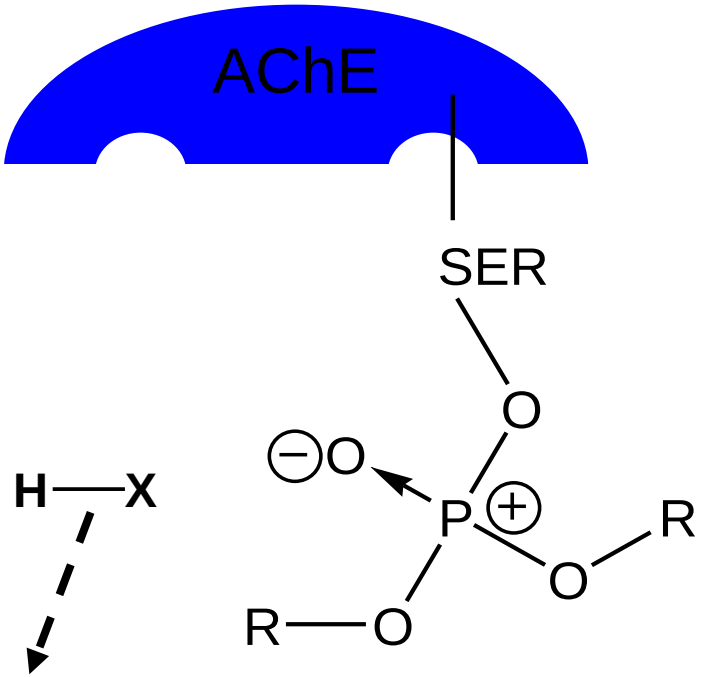
Oxidation (activation) of aflatoxin B1
by cytochrome P450,
leading to liver tumor formation.

Oxidative deamination and desulfuration



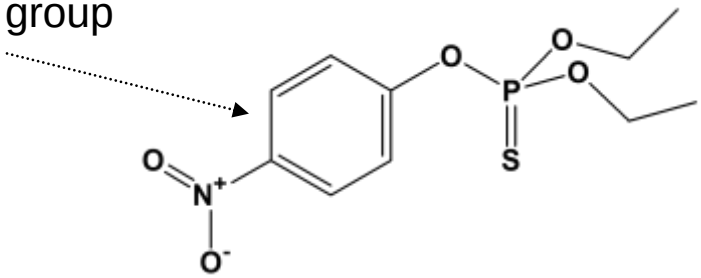
Oxidative desulfuration of OPs: Lethal synthesis





Phosphorylated AChE

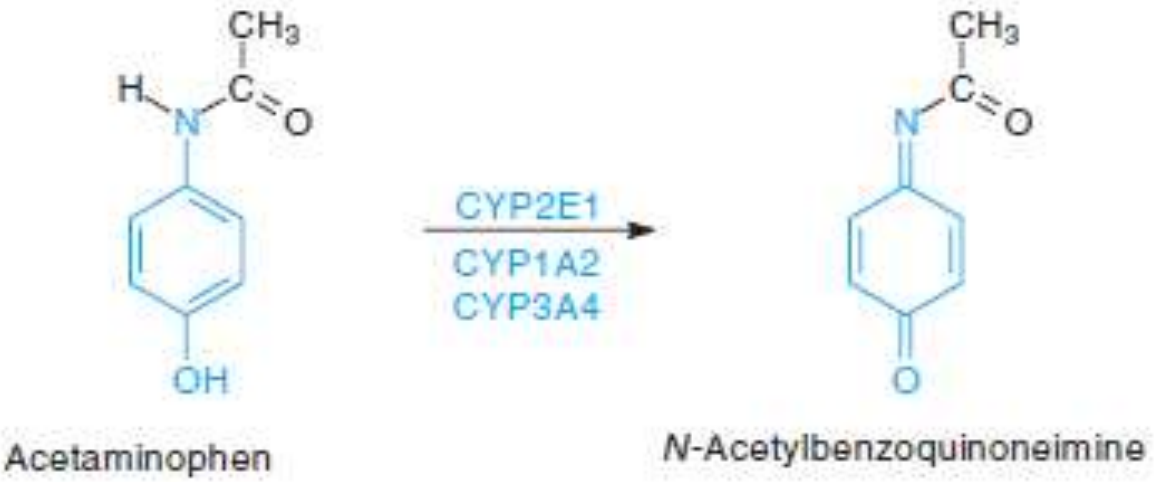
X = Leaving group



Parathion

Specific mode of action:
inhibition of acetylcholinesterase
by oxon metabolite

Dehydrogenation leading to formation of toxic metabolite

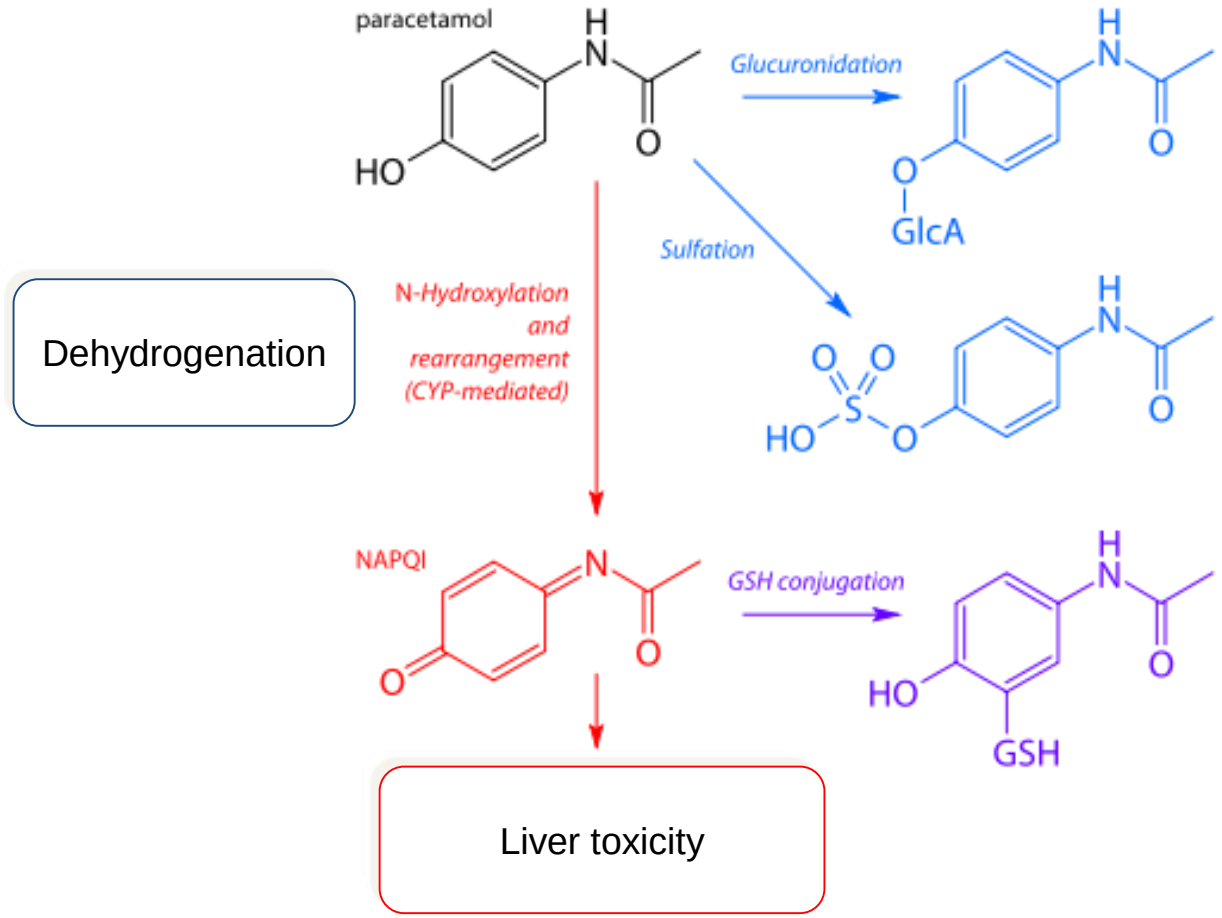


Acetaminophen

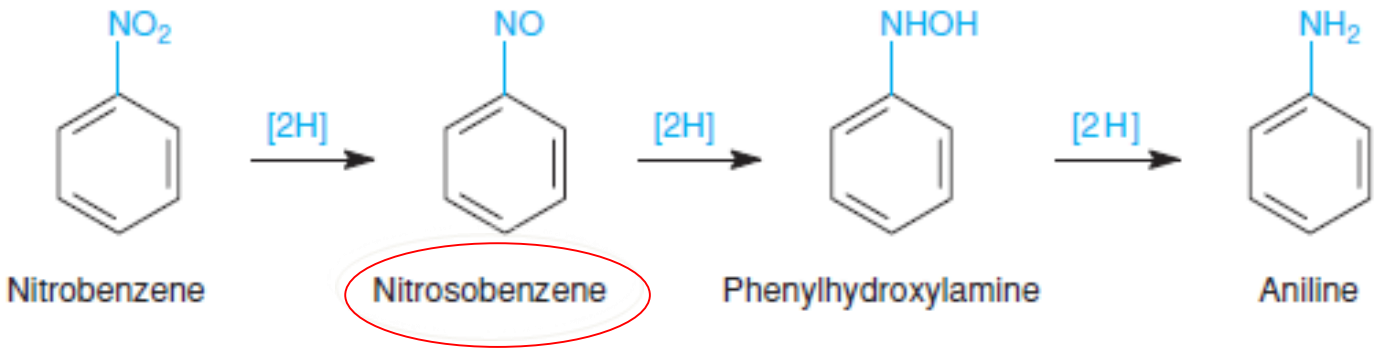
N-Acetylbenzoquinoneimine

In acetaminophen overdose, N-acetylbenzoquinoneimine (NAPQI) may act directly (covalently bind) on liver macromolecules, leading to hepatotoxic effects.

Acetaminophen (paracetamol) metabolism vs. toxicity

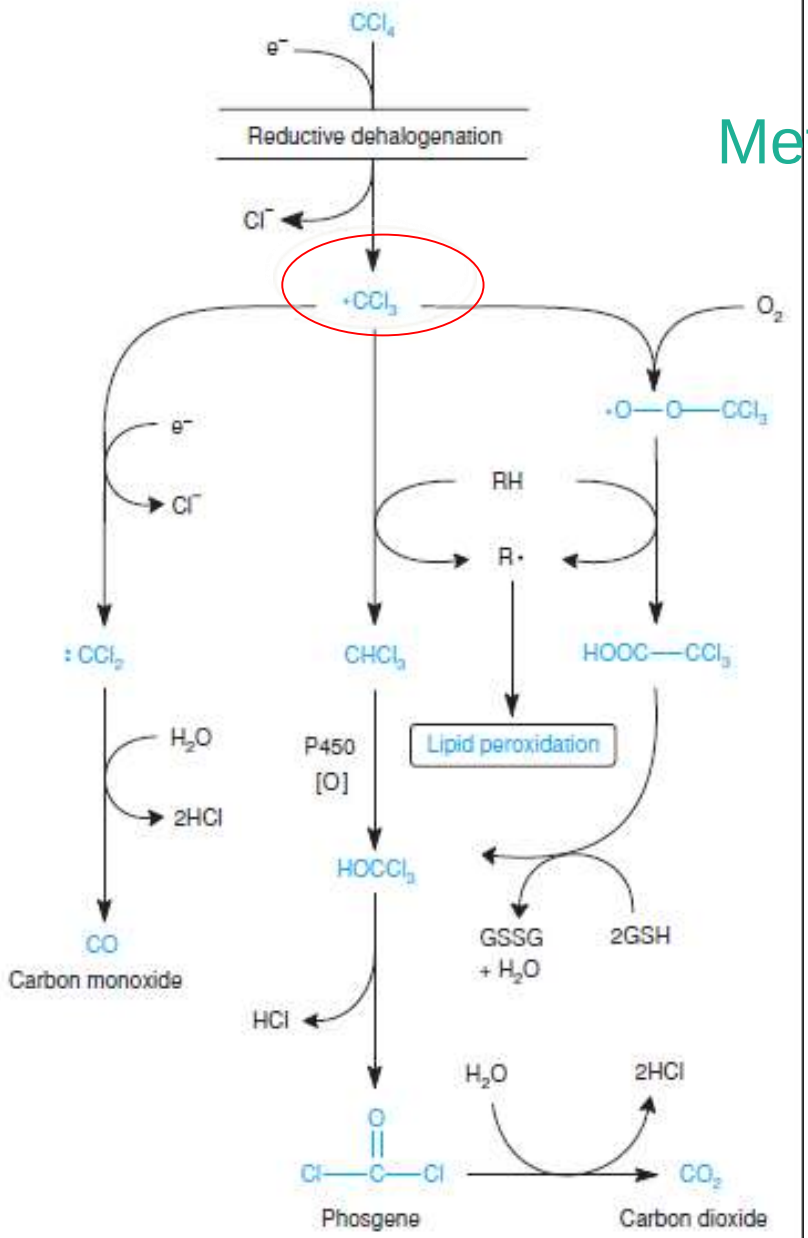


Reduction *vs.* toxicity



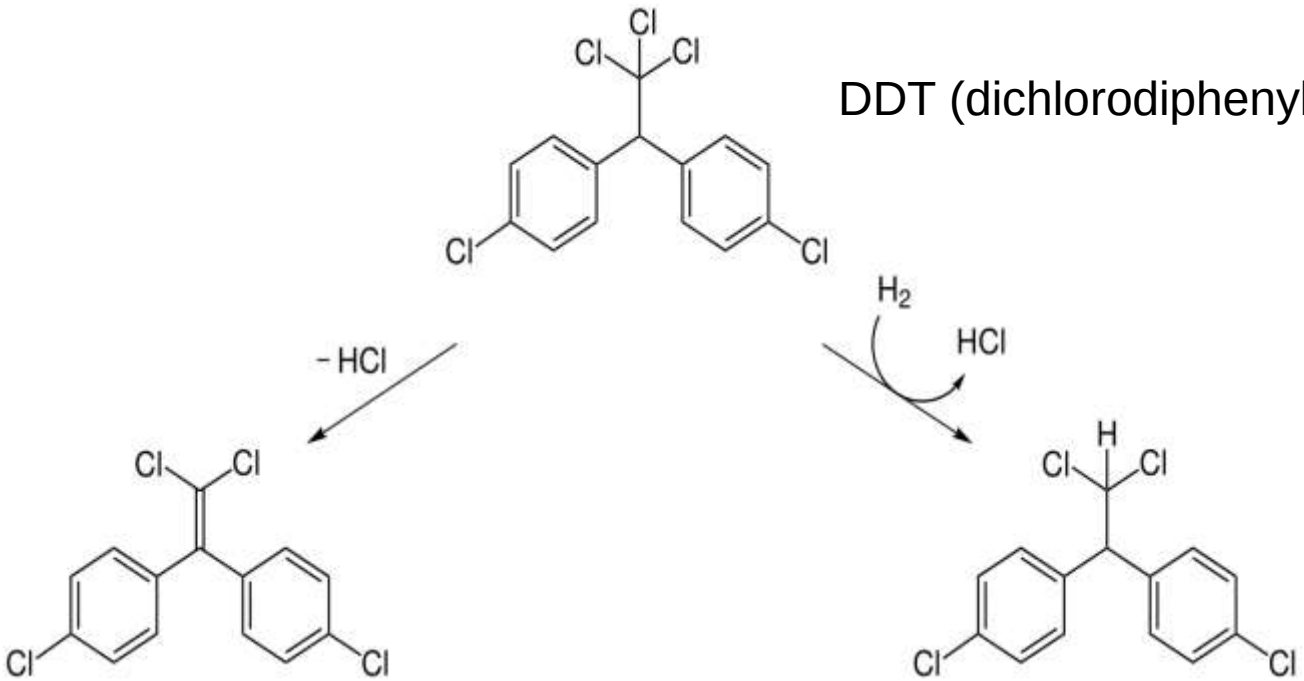
Nitroso- group is an alert structure.

Metabolism *vs.* mechanism of toxicity



Reductive dehalogenation of carbon tetrachloride to a trichloromethyl free radical that initiates lipid peroxidation and liver toxicity.

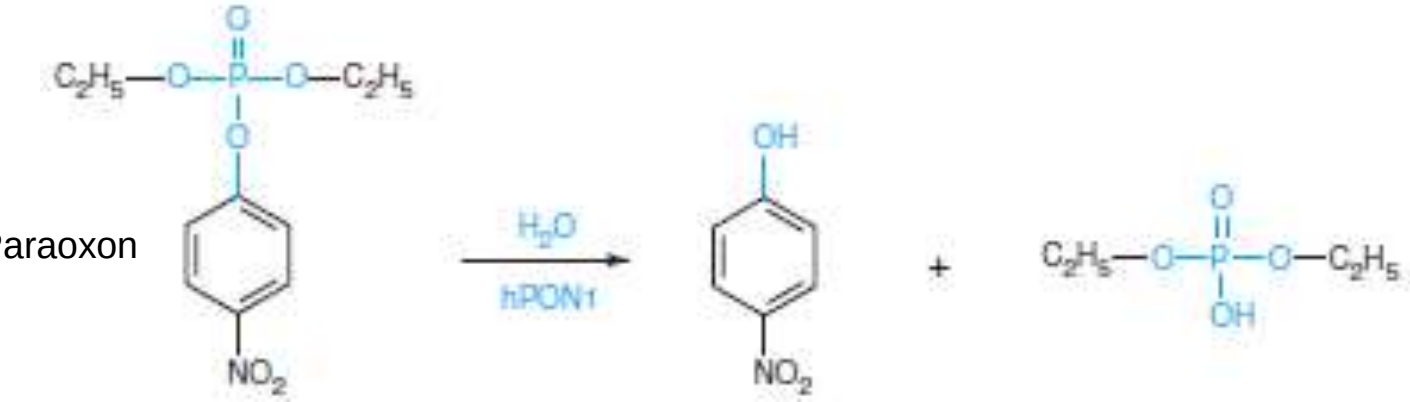
Dechlorination of the pesticide DDT

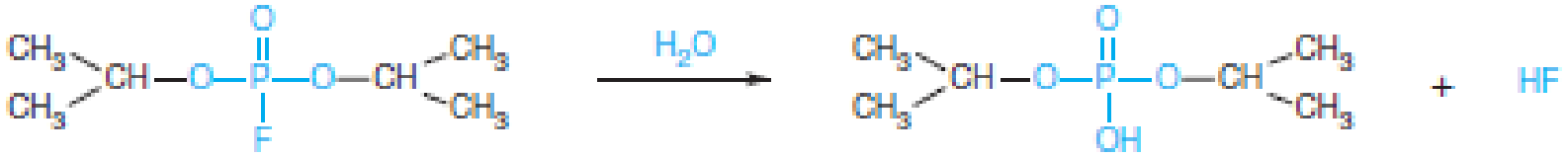


Degradation of DDT to form DDE (dehydrochlorination) and DDD (by reductive dechlorination).

Hydrolysis of OPs

Paraoxon

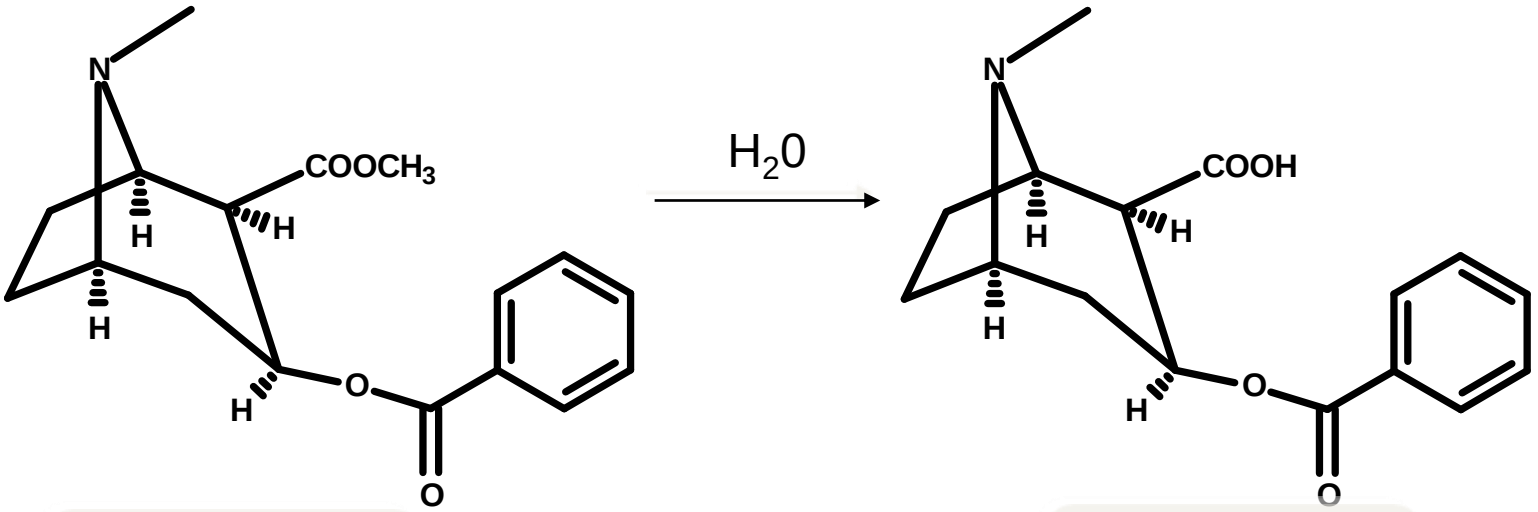




Diizopropylfluorophosphate (DFP)

Hydrolysis is detox mechanism in OPs exposure.
Antidotal therapy should be given as long as metabolites are present in urine.
Six urinary dialkyl phosphate (DAP) metabolites of OPs insecticides are accepted as biomarkers of OPs exposure.

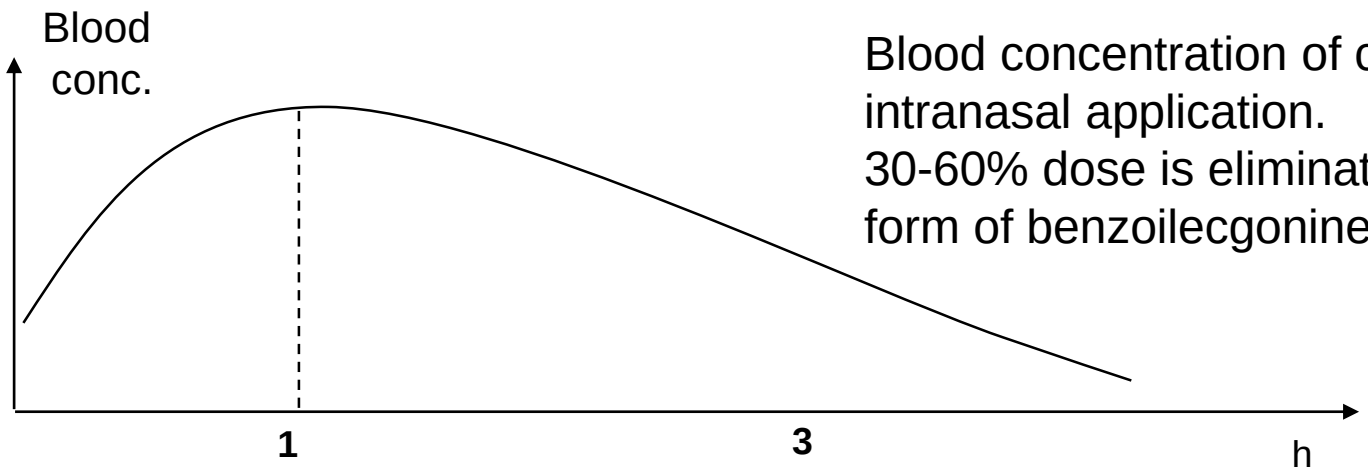
Hydrolysis of cocaine



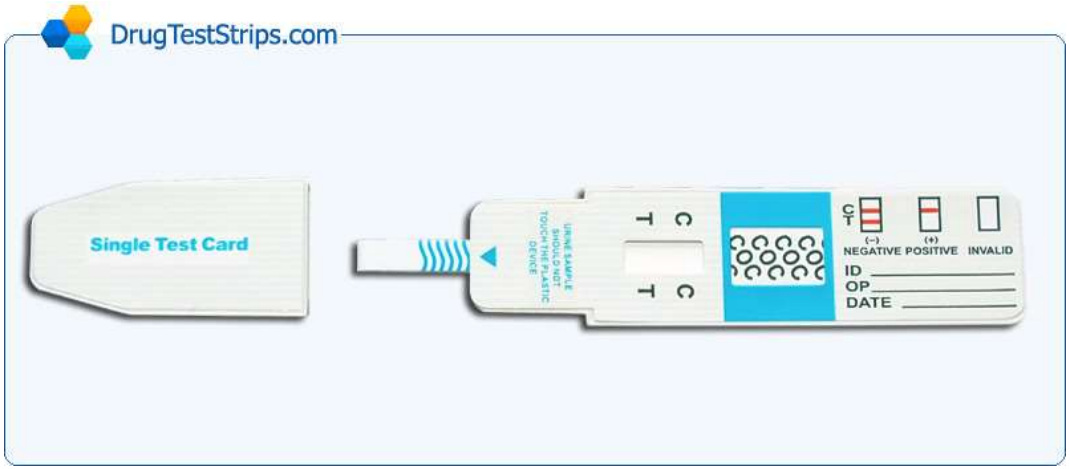
Cocaine
Active

Benzoilecgonine
Inactive

Rapid hydrolysis of cocaine

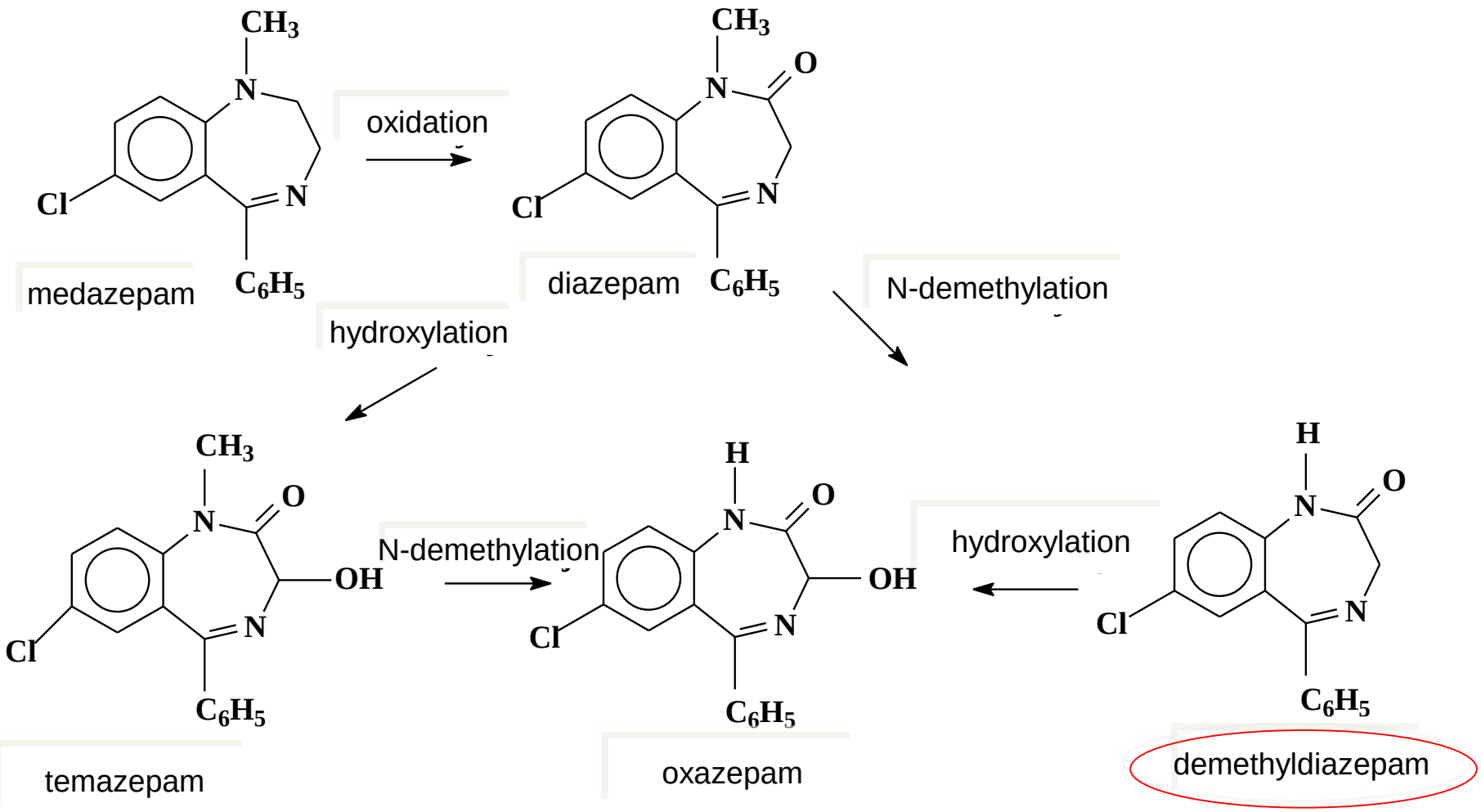


Blood concentration of cocaine after intranasal application.
30-60% dose is eliminated by urine in a form of benzoilecgonine.



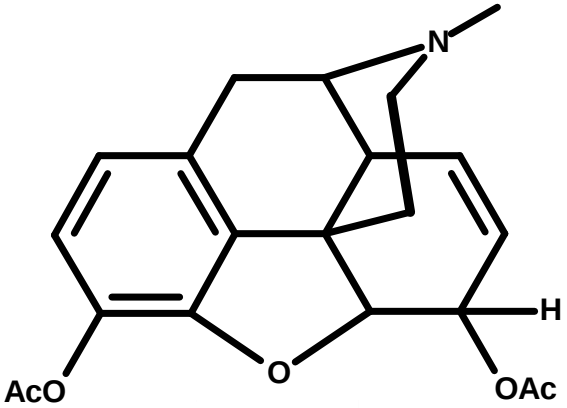
Test strip for detection of cocaine in urine in a form of benzoilecgonine.

Metabolism of benzodiazepines



glucuronide conjugation

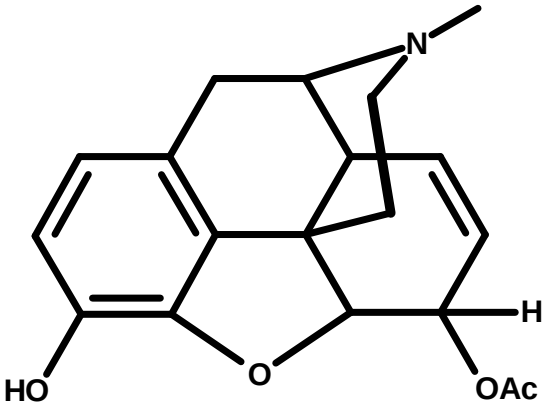
Metabolism of heroine



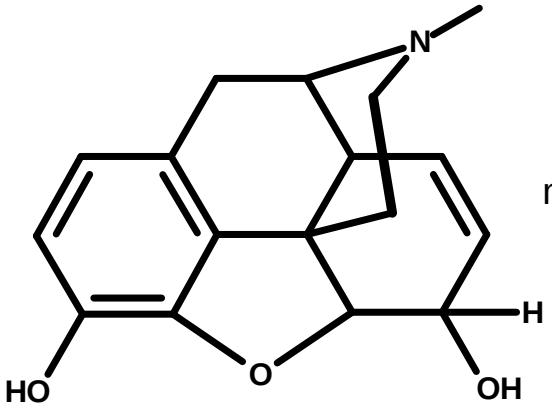
heroine

- heroine $t_{1/2}$ plasma - 3 min
- morphine $t_{1/2}$ plasma - 2-3 h
- 24 h urine elimination - 80 % dose
- unchanged heroine in urine - 0,1%

deacetylation



6-monoacethylmorphine

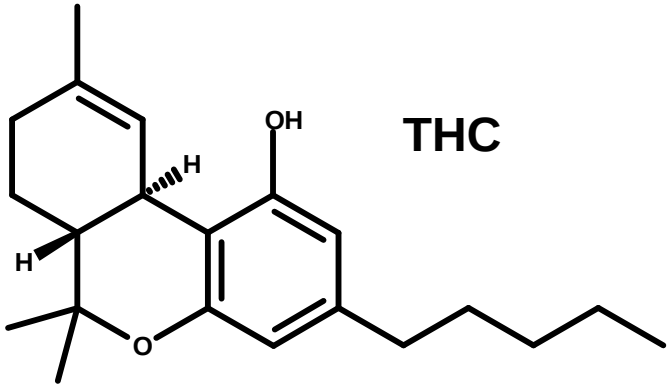


morphine

glucuronide conjugation

Metabolism of THC

- Cannabinol, cannabidiol (inactive)
- **THC** *trans*-Δ9-tetrahydrocannabinol (active)



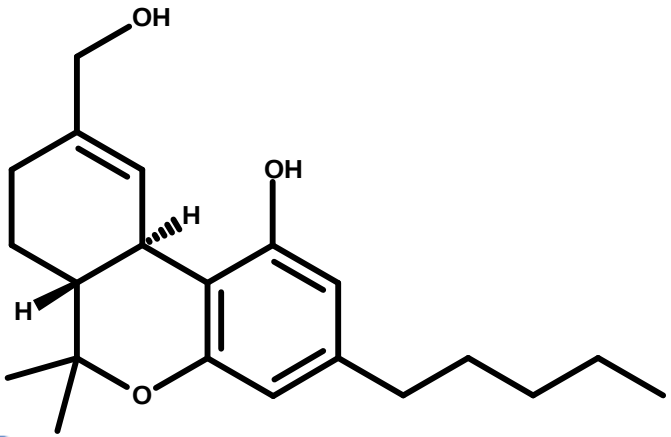
THC

THC → 11-hidroksi-delta-9-THC (active) →

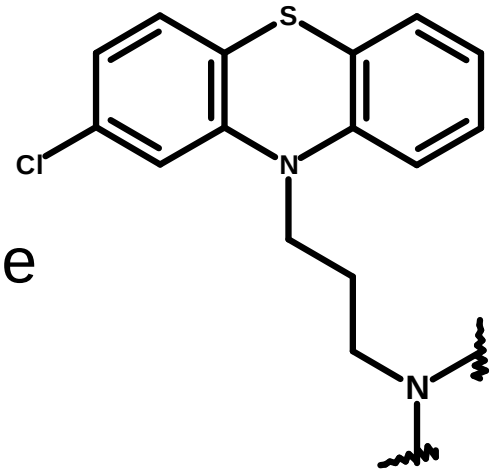
[O]

→11-nor-delta-9-THC-delta-9-carbonic acid →

→ glucuronide



Metabolism of phenotiazines



chloropromazine

- Known for its huge number of metabolites (around 150)
- N-oxidation
- Sulfoxidation
- N-demethylation
- conjugation

Xenobiotic biotransformation can alter the biological properties of a xenobiotic

Phase I	Phase II
Active to Inactive	
Amphetamine (P450)→Phenylacetone Cocaine (esterase) → benzoylecgonine	Aflatoxin 8,9-epoxide (GST) → 8-glutathionyl-9-hydroxyaflatoxin
Active to Active	
Acetylsalicylic acid (esterase) → Salycylic acid Heroin (esterase) → Morphine	Morphine (UGT) → Morphine-6-glucuronide
Inactive to Toxic (Lethal synthesis)	
Acetaminophen (P450) → N-acetyl-p-benzoquinine imine Malathion (P450) → Malaoxon Aflatoxin (P450) → Aflatoxin-8,9-epoxide	

Conclusions

Why/When do we need to understand biotransformation of poisons?

- When the delivered metabolite is acknowledged to be the primary active entity.
- When the compound is metabolised to one or more toxicologically active metabolites which could make a significant contribution to tissue/organ responses.
- When measurement of metabolite concentrations in plasma or other body fluids is especially important in the conduct of toxicokinetics.
- When measurement of metabolite concentrations in plasma or other body fluids is especially important for the efficient antidotal and other therapy.
- When the administered compound is very extensively metabolised and the measurement of plasma or tissue concentrations of a major metabolite is the only practical means of estimating exposure following administration of the compound in toxicity studies.

THANK YOU FOR YOUR ATTENTION!