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Biostransformation of xenobiotics

general principles

Tuesday, January 28th 2025

Kévin HARDONNIÈRE

M1D2HP_TU11_2024-2025

Objectifs

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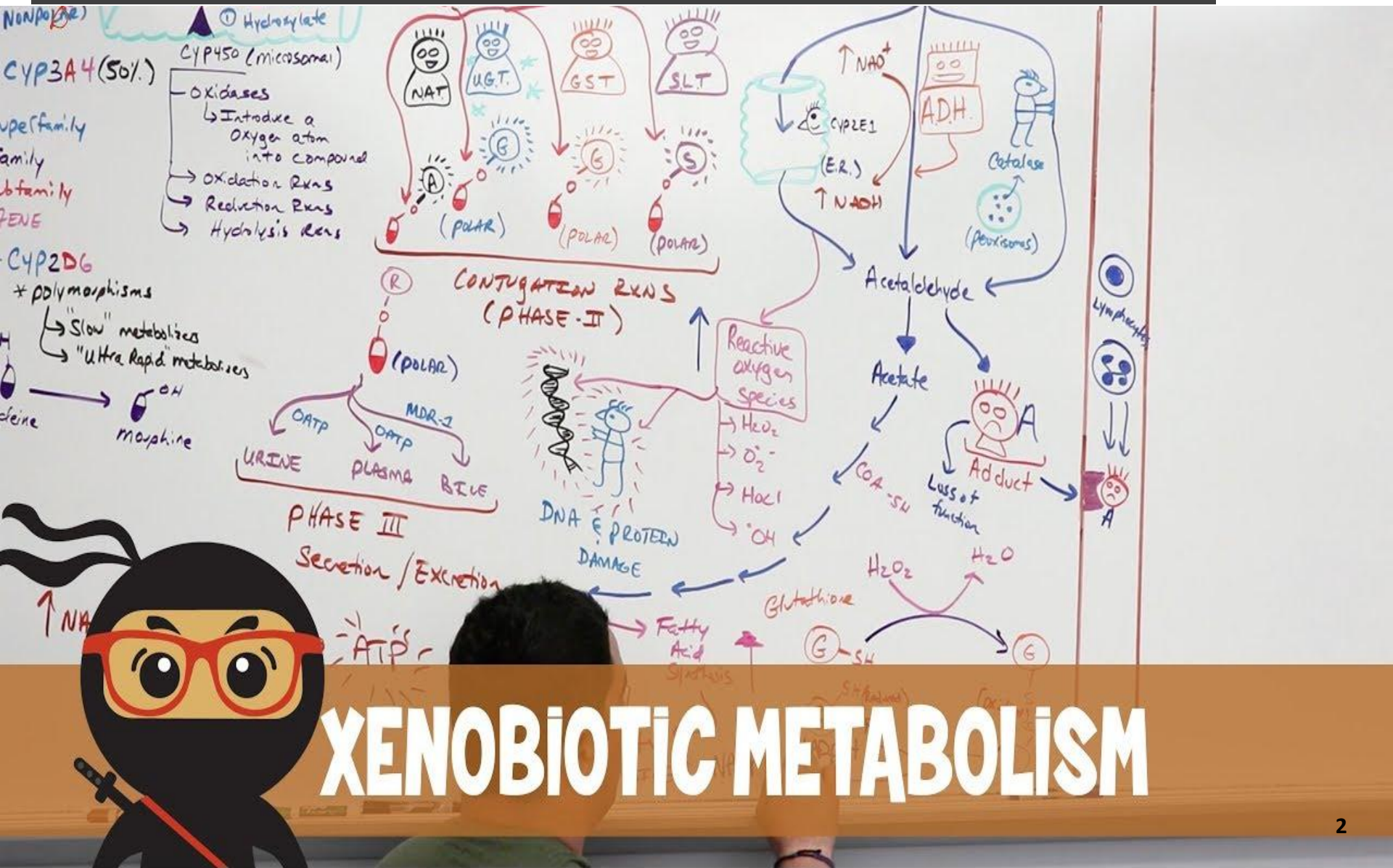

Basics

Definition

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Xénobiotic

The “xeno” in “xenobiotics” comes from the Greek word *xenos* meaning guest, friend, or foreigner.

Molecule of foreign origin to an organism, present exceptionally in the organism and which is neither a substrate, nor a usual product of metabolic reactions.

These low molecular weight molecules are very varied in nature; they include, for example, food contaminants, synthetic compounds or their by-products, drugs and environmental pollutants.

Properties of xenobiotics

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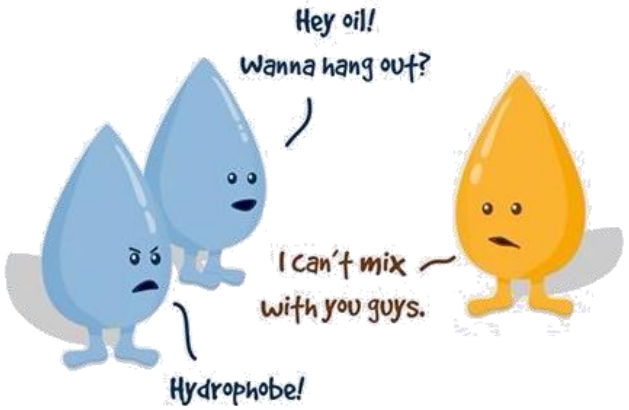
- ✓ These compounds are rather lipophilic;
- ✓ **Passage of membres** per diffusion;
- ✓ Carried by **lipoproteins** in the blood.



A chemical transformation is required to allow their elimination !

What do we mean by biotransformation

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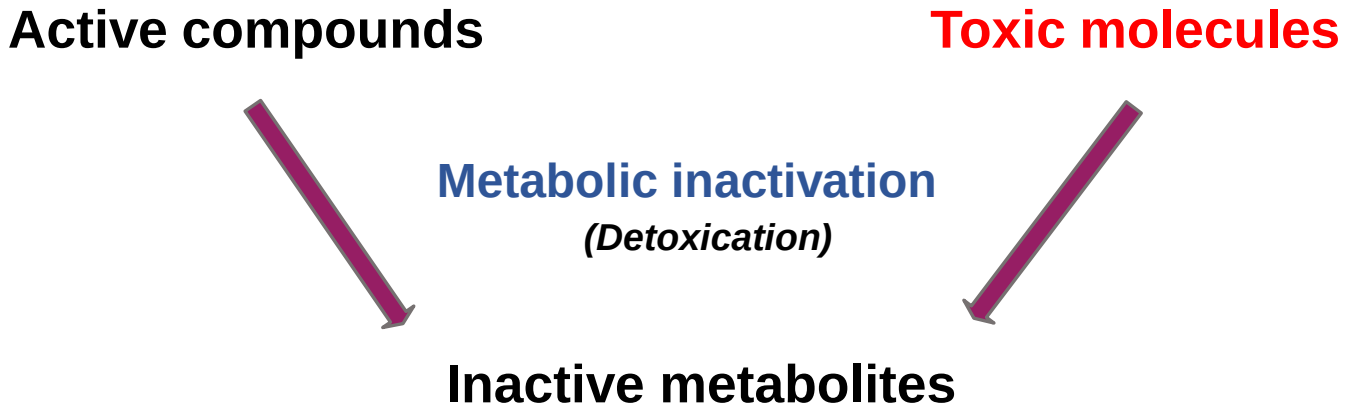
- ✓ The term "biotransformation" refer to the **chemical modifications** that these molecules will encounter within the body to generate some **new metabolites.**

- ✓ Biotransformations are mainly carried out by **enzymatic reactions**.

- ✓ The main function of biotransformations is therefore **to make lipophilic molecules water-soluble** in order to **promote their elimination from the body.**

Metabolic inactivation



Metabolic activation

Prodrugs → **Active metabolites**

Non toxic compounds → **Reactives metabolites**

Metabolic activation

Basics on xenobiotics metabolism

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- ✓ Metabolism of xenobiotics most of the time helps to **protect the body upon exposure**;

- ✓ It is therefore essential to allow the **elimination of toxic molecules**;

- ✓ The process is divided in **2 steps** in order to transform the xenobiotic into **a more hydrophilic metabolite**, thus **facilitating its body elimination**;

Basics on xenobiotics metabolism

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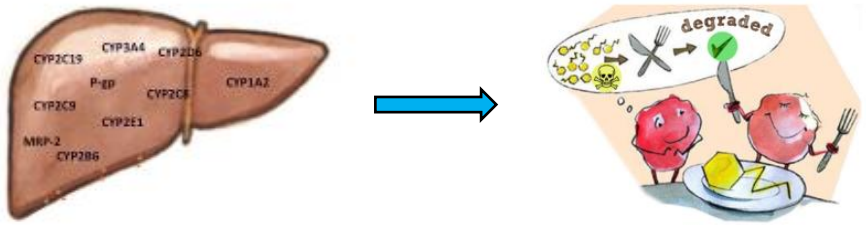
The liver is a key organ in the metabolism of drugs and xenobiotics

- ✓ In addition to its metabolic capacity, the liver is also involved in the excretion of drugs from the body through the biliary system. After excretion in the bile, the drug ends up in the intestinal lumen where it can be reabsorbed: this is **the enterohepatic cycle**.

- ✓ The xenobiotics absorbed at the intestinal level will be preferentially **processed in the liver** before entering the general circulation (**first-pass effect**, **enterohepatic cycle**) but also in the kidneys, the digestive tract, the lungs, the skin…etc.



Overview at the cellular level…

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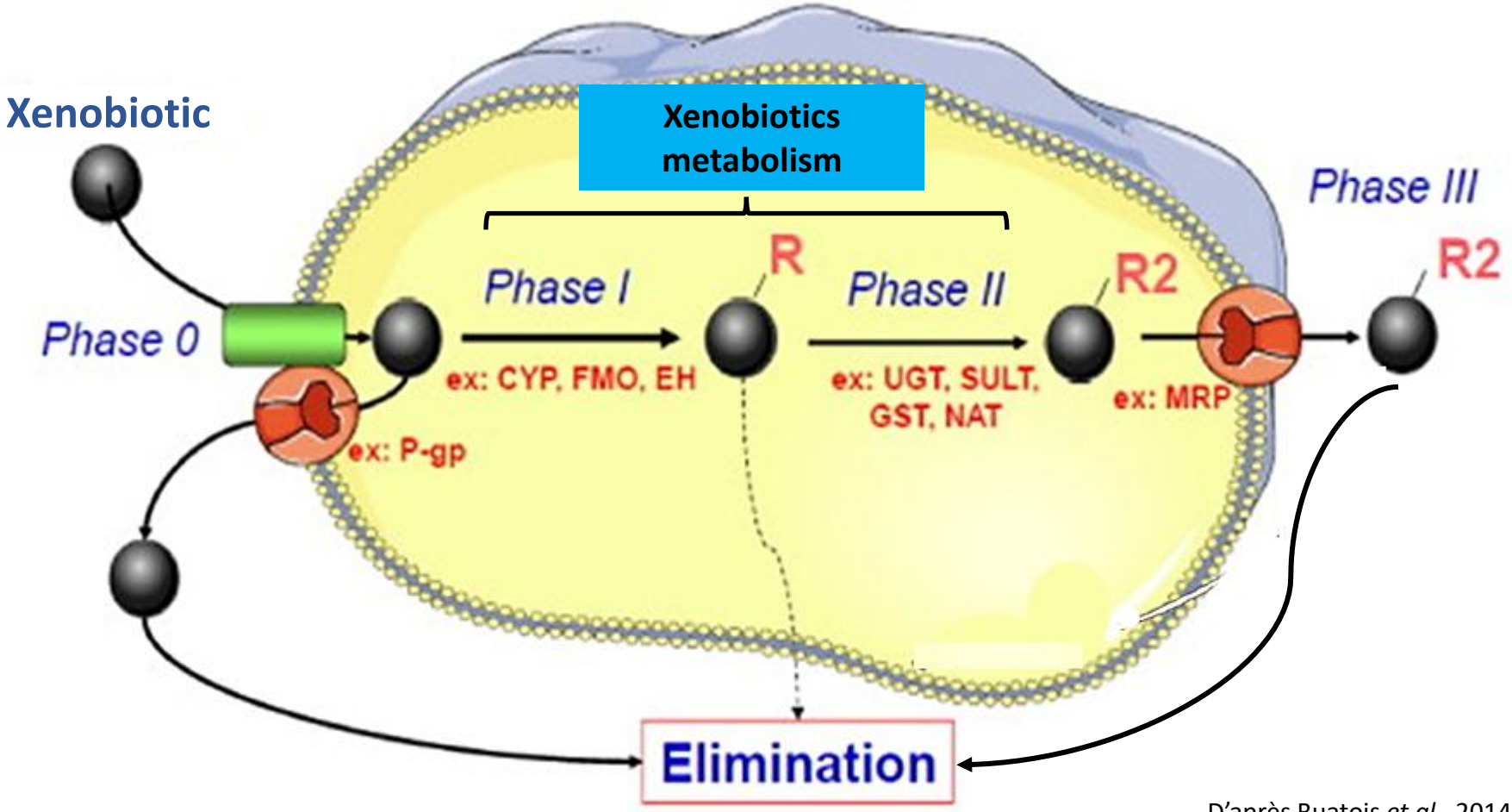

D'après Buatois *et al.*, 2014

Sequential processing of xenobiotics according to their physicochemical properties

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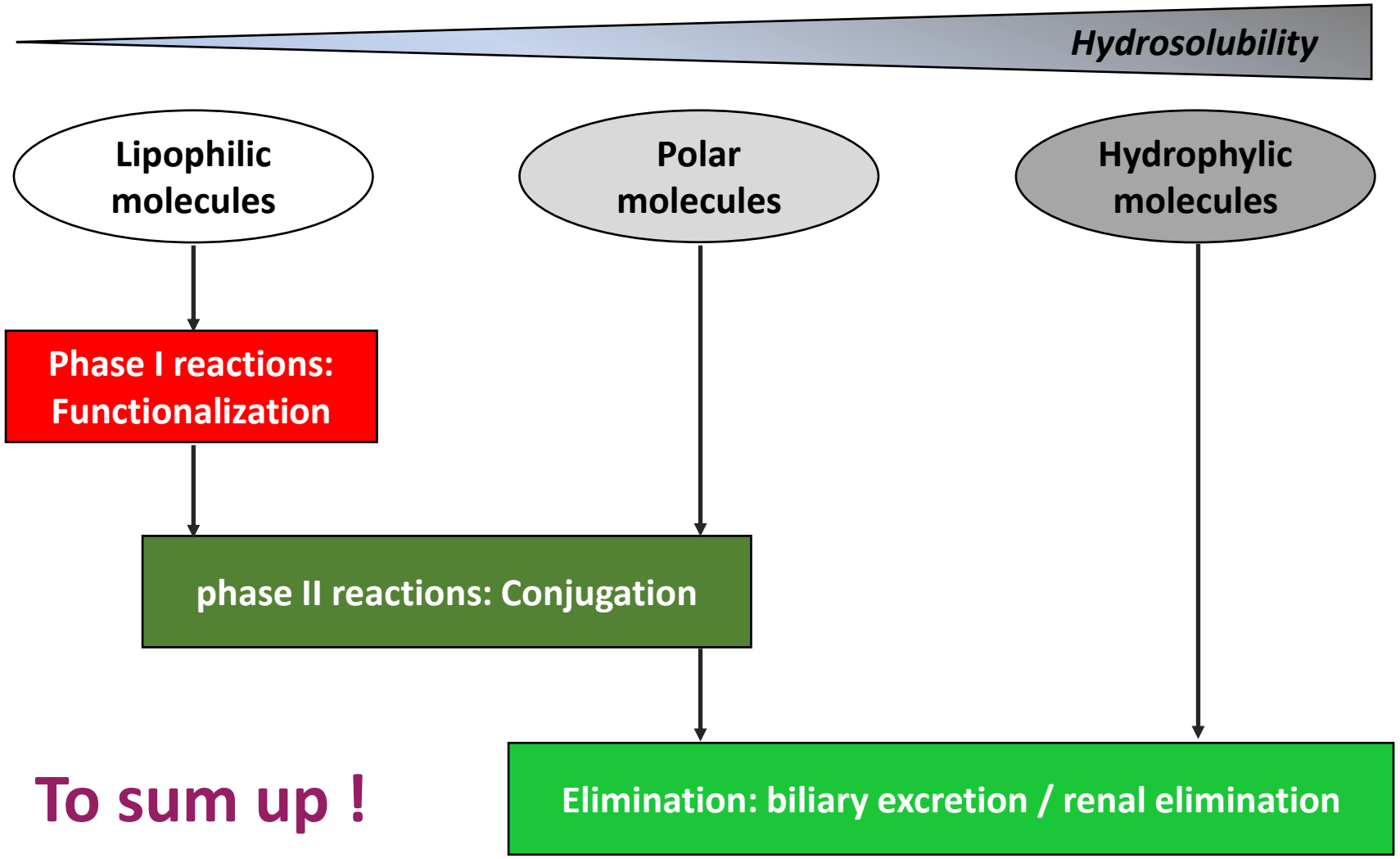

To sum up !

Phase I reactions

Fonctionnalisation : Phase I

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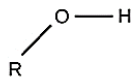
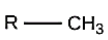
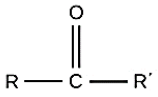
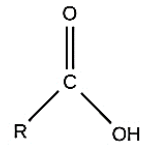
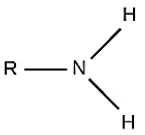
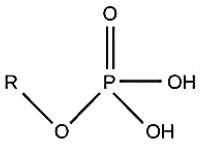
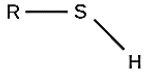
Creation of a functional group -OH, -NH_2, -COOH, -SH

Oxidations

+O_2 -H^+

Reductions

-O_2 +H^+

Functional Group	Structure	Properties
Hydroxyl		Polar
Methyl		Nonpolar
Carbonyl		Polar
Carboxyl		Charged, ionizes to release H^+. Since carboxyl groups can release H^+ ions into solution, they are considered acidic.
Amino		Charged, accepts H^+ to form NH_3^+. Since amino groups can remove H^+ from solution, they are considered basic.
Phosphate		Charged, ionizes to release H^+. Since phosphate groups can release H^+ ions into solution, they are considered acidic.
Sulfhydryl		Polar

Hydrolysis

+ H_2O

Covalent bond disruption

Hydratations

+ H_2O

Electrophilic addition of a molecule of water to an organic compound

General pathways and their major cellular location

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REACTION	ENZYME	LOCALIZATION
	Phase I	
1 *Hydrolysis*	Esterase	Microsomes, cytosol, lysosomes, blood
	Peptidase	Blood, lysosomes
	Epoxide hydrolase	Microsomes, cytosol
2 *Reduction*	Azo- and nitro-reduction	Microflora, microsomes, cytosol
	Carbonyl reduction	Cytosol, blood, microsomes
	Disulfide reduction	Cytosol
	Sulfoxide reduction	Cytosol
	Quinone reduction	Cytosol, microsomes
	Reductive dehalogenation	Microsomes
3 *Oxidation*	Alcohol dehydrogenase	Cytosol
	Aldehyde dehydrogenase	Mitochondria, cytosol
	Aldehyde oxidase	Cytosol
	Xanthine oxidase	Cytosol
	Monoamine oxidase	Mitochondria
	Diamine oxidase	Cytosol
	Prostaglandin H synthase	Microsomes
	Flavin-monooxygenases	Microsomes
	Cytochrome P450	Microsomes

Phase I-mediated hydrolysis

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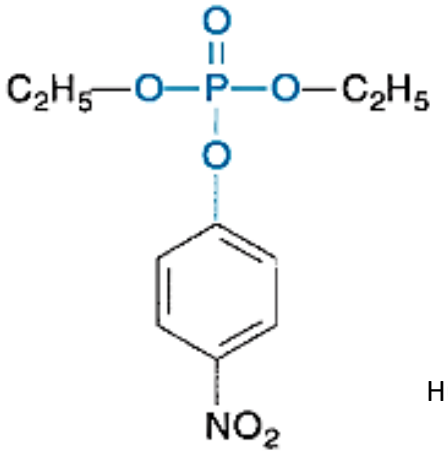
Breaking of a covalent bond by the action of a water molecule

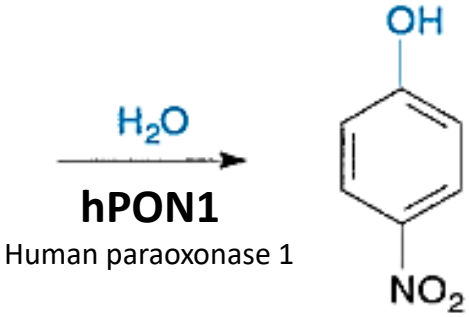
$$\text{R-COO-R' + } H_2O \Rightarrow \text{R-COOH + R'-OH}$$

$$\text{R-CO-NH-R' + } H_2O \Rightarrow \text{R-COOH + R'-}NH_2$$

Réactions d'hydrolyses :



Paraoxone



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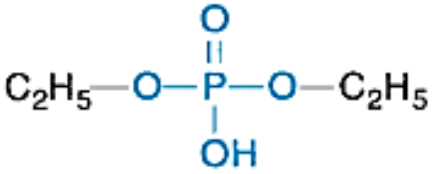

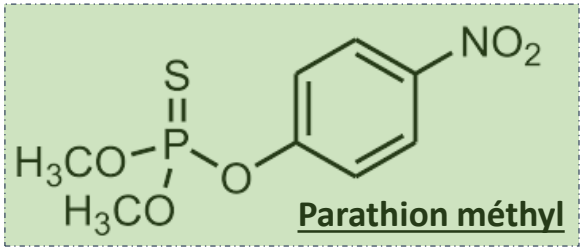

Epoxide Hydrolase (EH)

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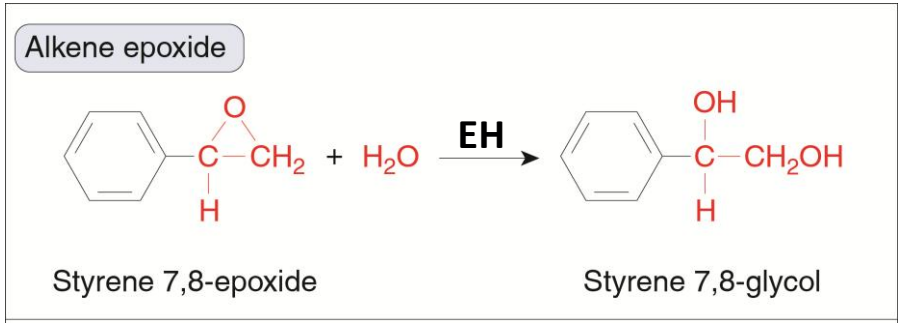
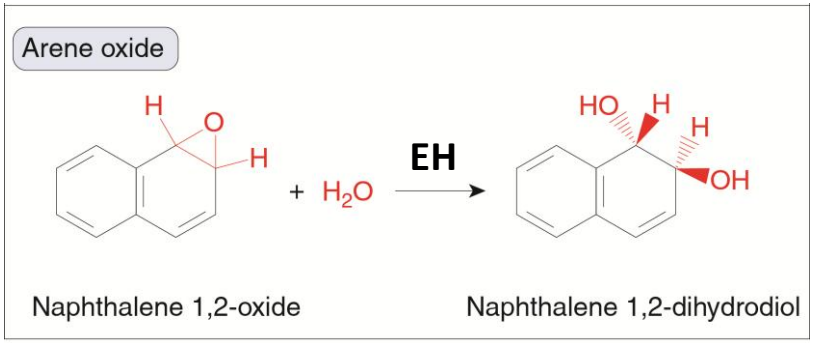
1

- ✓ **5 isoformes in human;**
- ✓ **Microsomal and cytoplasmic enzymes;**
- ✓ **Conversion of epoxy groups into dihydrothiol groups;**
- ✓ **Detoxification of molecules with reactive epoxy groups capable of forming adducts (covalent bonds) on proteins and nucleic acids.**

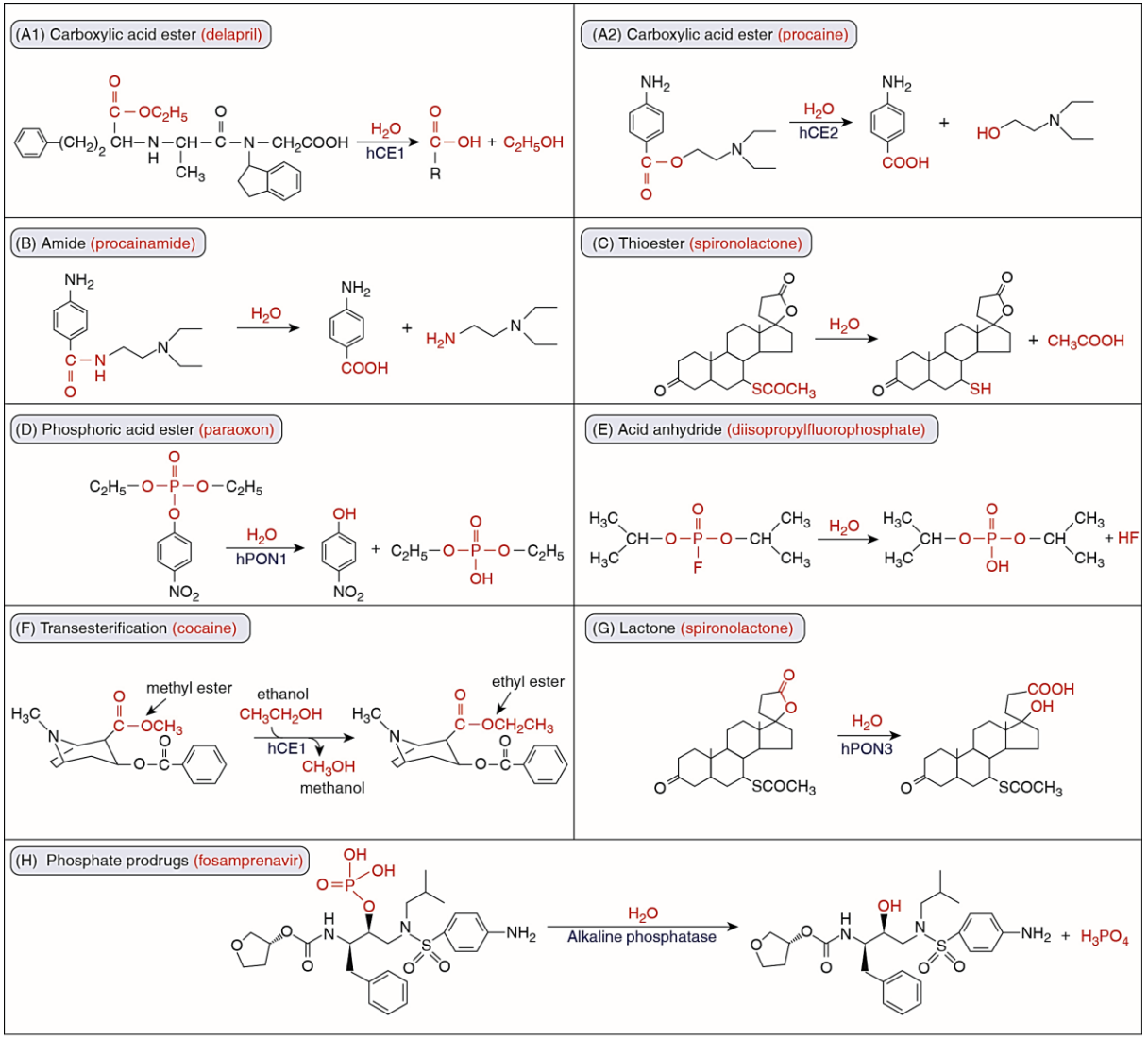




Examples of phases I hydrolysis reactions

1

Carboxylesterases, cholinesterases and organophosphatases



Phase I reduction reaction

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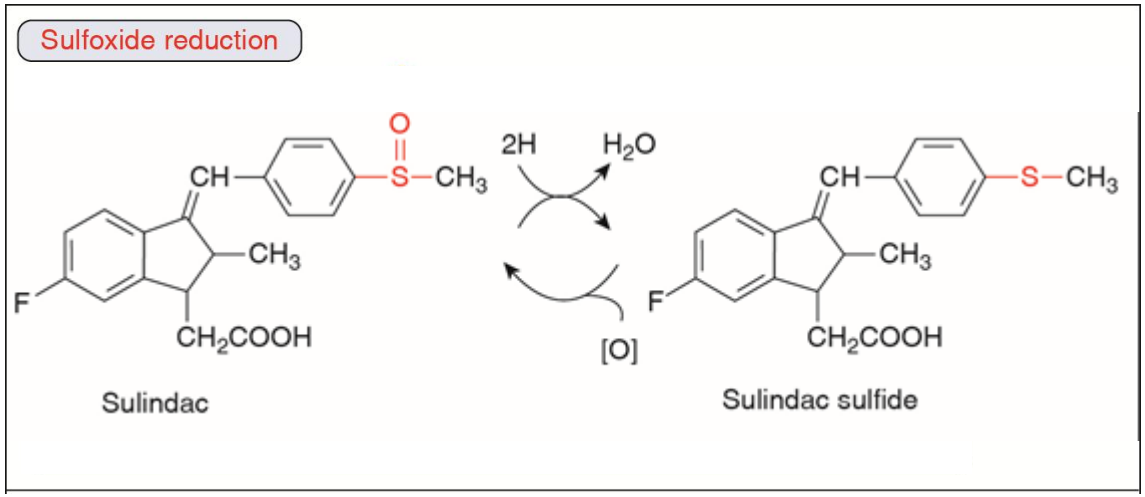
Adding a proton
or removing an oxygen atom

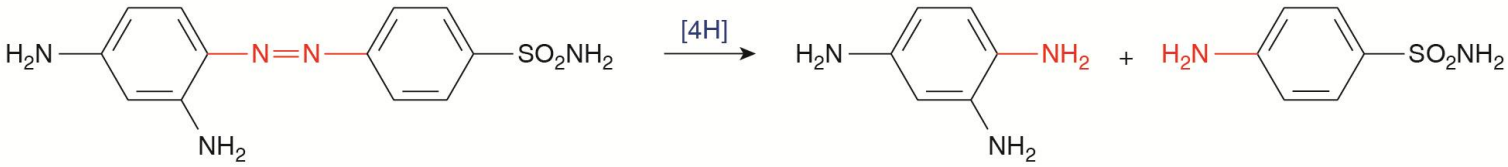
- **R-OH ⇒ R-H** (Dehydroxylation)
- **R-C=O ⇒ R-C-OH** (Hydrogenation)
- **R-COOH ⇒ R-C=O** (Decarboxylation)
- **R-NO_2⇒ R-NH_2** (Amination)
- **R-C-H ⇒ R-CH_3** (Methylation)



Others examples of phase I reductions

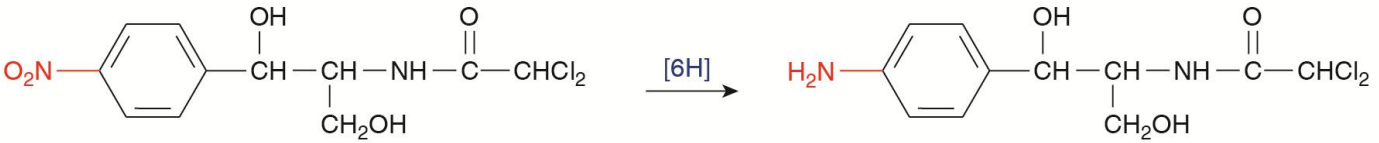
2

Azo-reduction

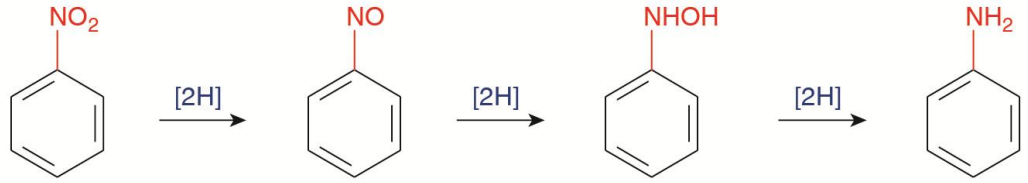


Prontosil — 1,2,4-Triaminobenzene — Sulfanilamide

Nitro-reduction



Chloramphenicol — Arylamine metabolite



Nitrobenzene — Nitrosobenzene — Phenylhydroxylamine — Aniline

Phase I oxidations

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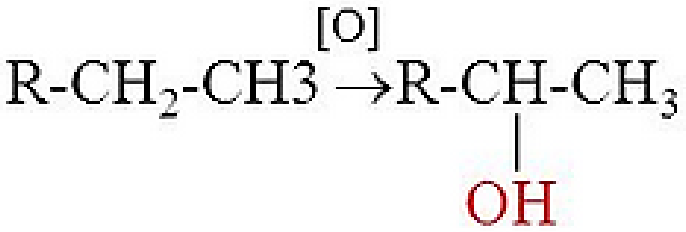
3

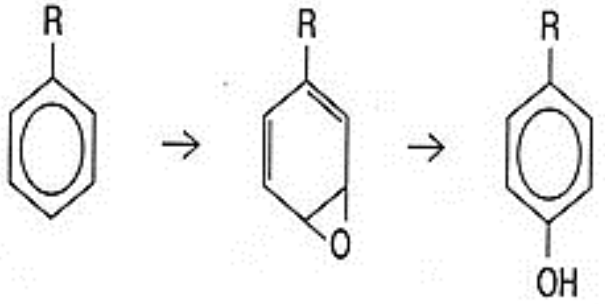
Addition of an oxygen molecule or loss of a proton

- **R-H ⇒ R-OH** (Hydroxylation)
- **R-C-OH ⇒ R-C=O** (Dehydrogenation)
- **R-C=O ⇒ R-COOH** (Carboxylation)
- **R-C-NH_2⇒R-C=O** (Deamination)
- **R-CH_3⇒ R-H** (Demethylation)

Aliphatic oxidation



Oxydations aromatiques



These are the most common Phase I reactions

Examples of phase I oxidations

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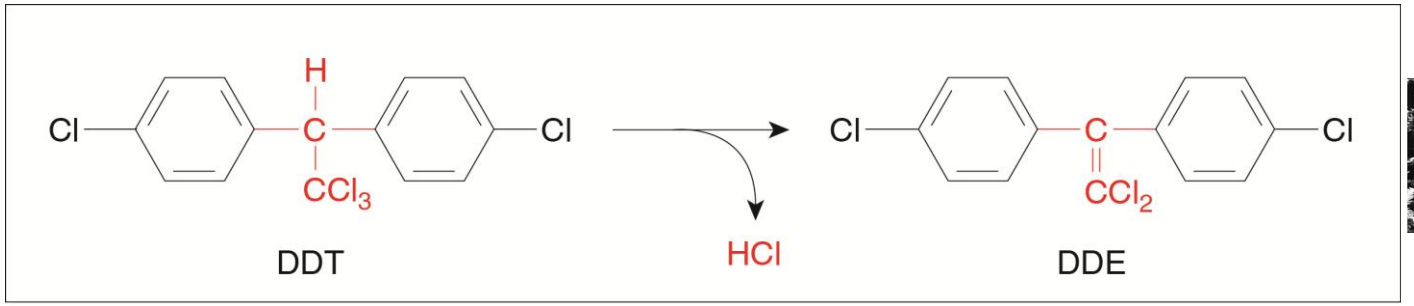
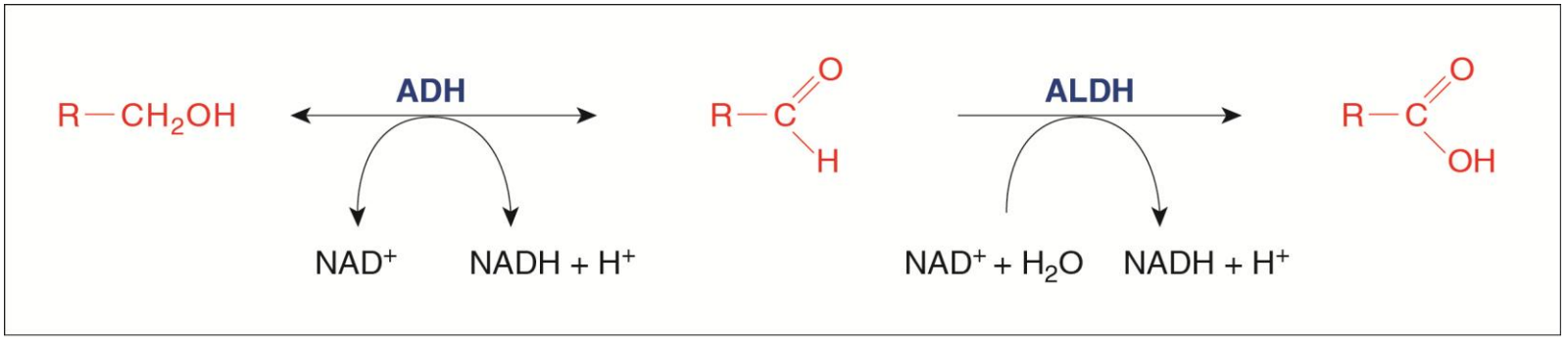
Dehydrochlorination of the pesticide DDT to DDE



Dichlorodiphenyltrichloroethane

Dichlorodiphenyldichloroethylene

Oxidation of alcohols to aldehydes and carboxylic acid

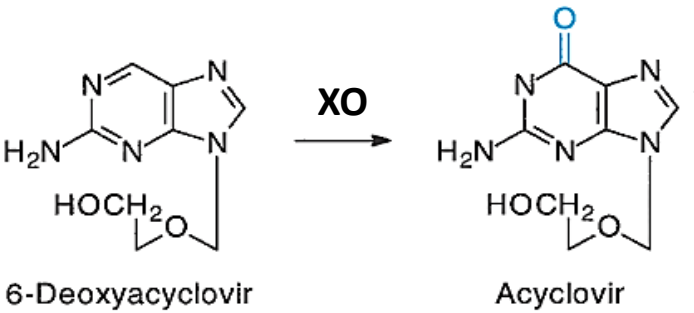


ADH = alcool dehydrogenase / ALDH = aldehyde deshydrogenase

Xanthines Oxidases (XO)

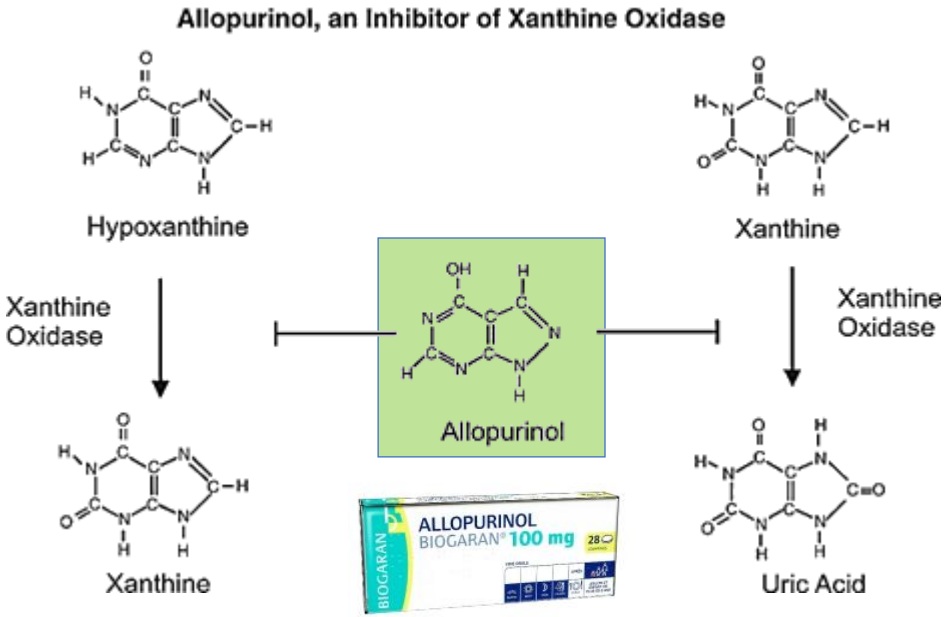
3

Prodrug activation



Krenitsky TA et al., 1984

Inhibition



↘ uricemia

Xanthinuria
XO deficiency

P450 cytochromes

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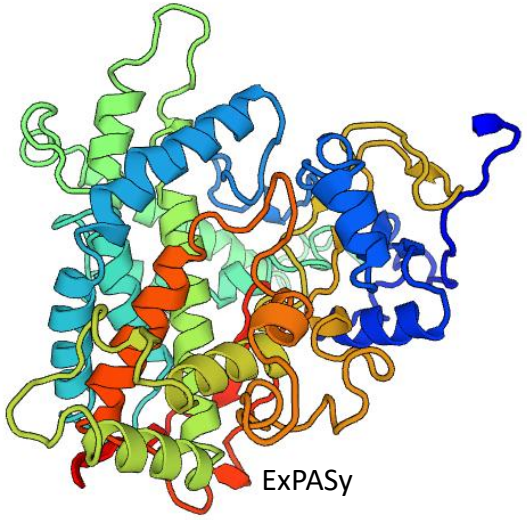
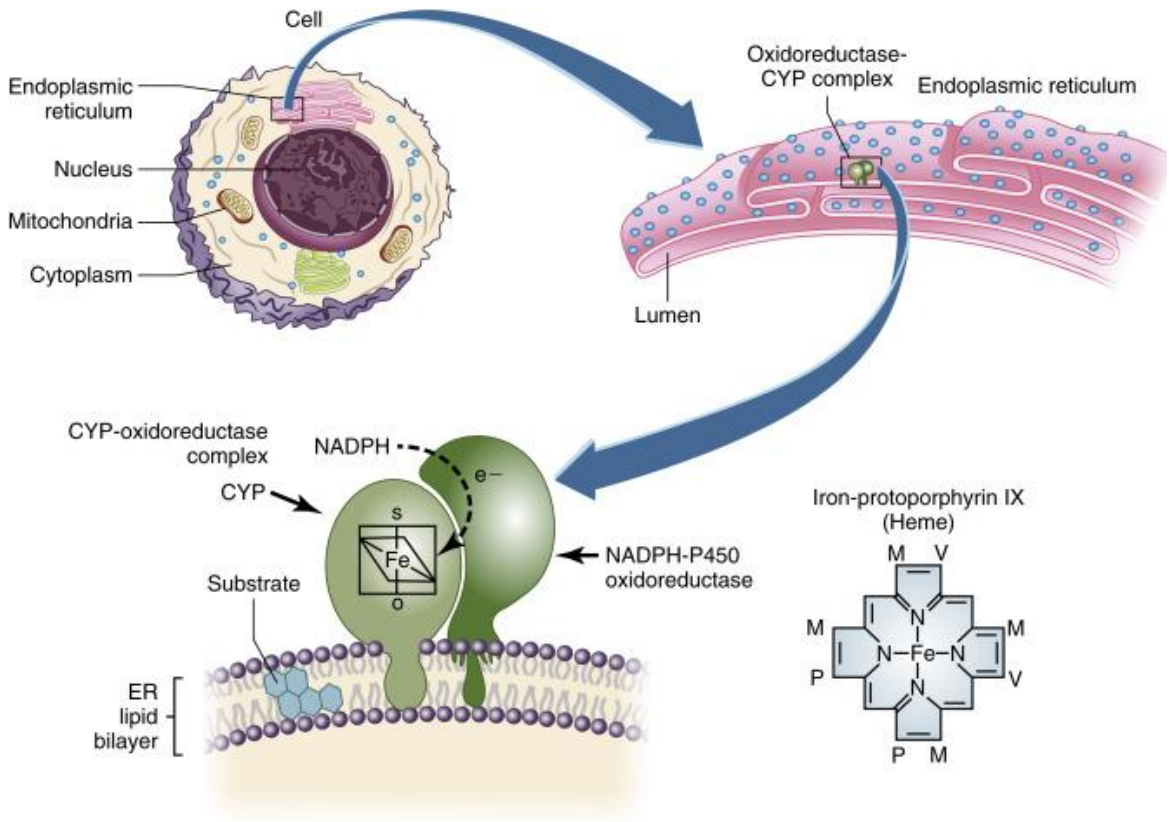
- ✓ **Pigment 450 = maximal absorption at 450 nm;**
- ✓ **Activité monooxygenase activity ;**
- ✓ **CYP450 catalyzes oxidation using oxygen and NADPH (reduced nicotinamide).**



Cytochrome P450 1A2

P450 localization



Localization in the membranes of the smooth ER of hepatocytes (Some CYPs are also localized to mitochondria) ;

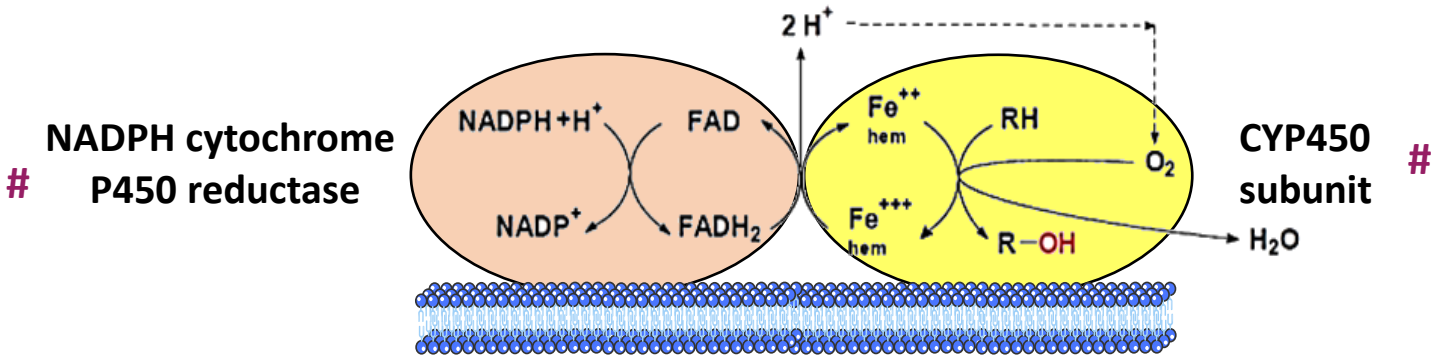
P450 monooxygenase activity

Three essential elements:

1°) Electron donors ➔ NADPH, NADH

2°) Electron transport chain #

3°) Phospholipids



$$RH + O_2 + NADPH + H^+ \rightarrow ROH + H_2O + NADP^+$$

Pentoses phosphates pathway, malic enzyme…etc.

Nomenclature

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	Codification	Example : CYP1A2
Cytochrome P450	CYP	CYP
Family	Number	1
Sub-family	Capital letter	A
Isoenzyme	Number	2

- ✓ ≈ 481 genes : 107 in human/ 57 characterized enzymes;
- ✓ Mainly expressed in the liver in mammals;

P450 substrates

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XENOBIOTICS		FATTY ACIDS/ EICOSANOIDS	STEROIDOGENIC	BILE ACIDS	VITAMIN D	RETINOIC ACID	UNKNOWN
CYP1A1	CYP2F1	CYP2U1	CYP11A1	CYP7A1	CYP2R1	CYP26A1	CYP4A22
CYP1A2	CYP2J2[*,†]	CYP4A11	CYP11B1	CYP7B1	CYP24A1	CYP26B1	CYP4X1
CYP1B1	CYP2S1	CYP4F2[‡,§]	CYP11B2	CYP8B1	CYP26C1[**]		CYP20A1
CYP2A6	CYP2W1	CYP4F3[§]	CYP17A1	CYP27A1[†]	CYP27B1		CYP27C1
CYP2A13	CYP3A4[†,††]	CYP4F8	CYP19A1	CYP39A1			
CYP2B6	CYP3A5	CYP4F11	CYP21A2	CYP46A1			
CYP2C8[*]	CYP3A7	CYP4F12[§]		CYP51A1[‡‡]			
CYP2C9[*]	CYP3A43	CYP4F22					
CYP2C18		CYP4V2					
CYP2C19		CYP4Z1					
CYP2D6		CYP5A1[§§]					
CYP2E1		CYP8A1[***]					

Note: CYP2A7 and 4B1 are full-length genes that probably encode inactive enzymes due to lack of heme incorporation.
[*] *Also involved in fatty acid and eicosanoid metabolism.*
[†] *Also involved in vitamin D metabolism.*
[‡] *Also involved in vitamin E and vitamin K metabolism.*
[§] *Also involved in xenobiotic metabolism.*
[**] *Also involved in retinoic acid metabolism.*
[††] *Also involved in bile acid synthesis.*
[‡‡] *Also involved in cholesterol biosynthesis.*
[§§] *Thromboxane A synthase (TBXAS1).*
[***] *Prostaglandin I_2 (prostacyclin) synthase (PTGIS).*

Importance of P450 in drug metabolism

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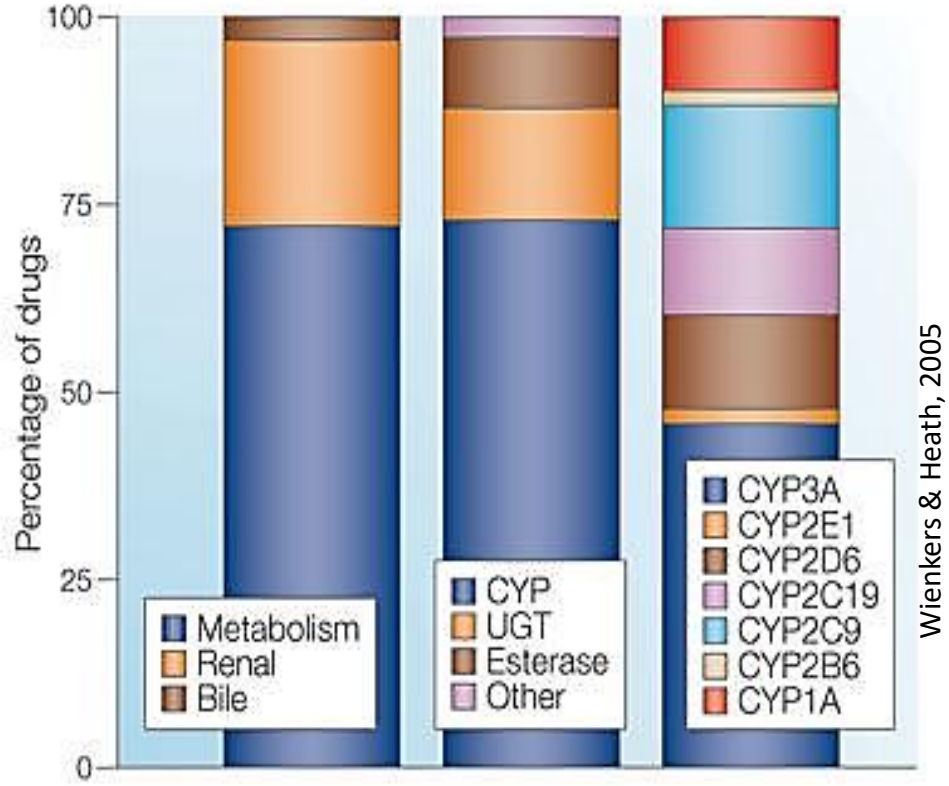
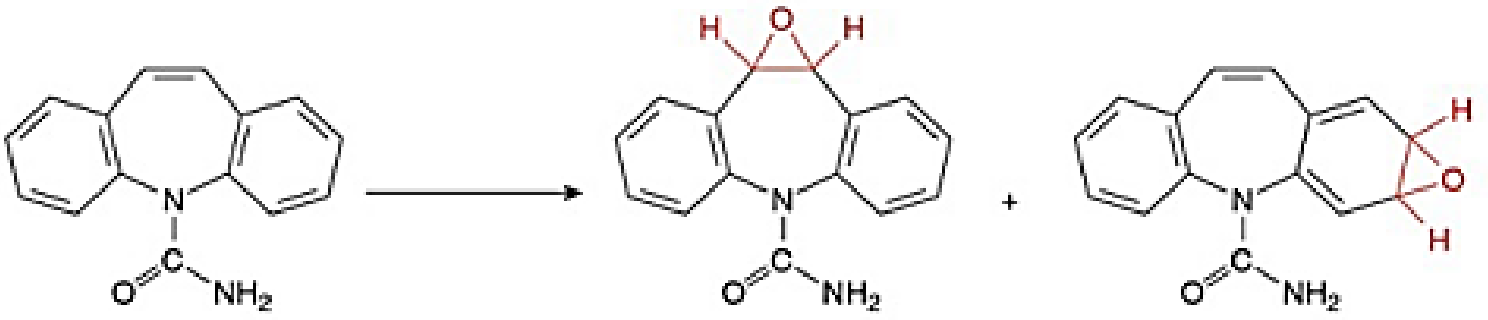
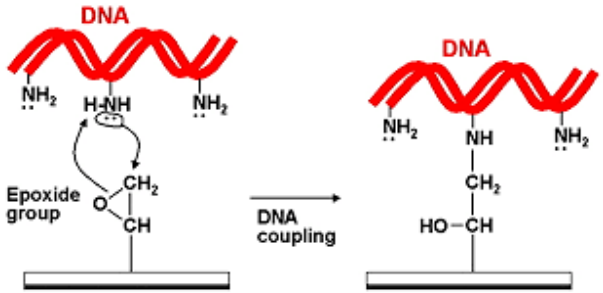


Elimination of the 200 most prescribed drugs

CYP P450 : epoxidation reactions





Generate reactive metabolites!

Phase II reactions

Phase II reactions

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Type of Conjugation	Endogenous Reactant	Transferase (Location)	Types of Substrates	Examples
Glucuronidation	UDP glucuronic acid	UDP glucuronosyltransferase (microsomes)	Phenols, alcohols, carboxylic acids, hydroxylamines, sulfonamides	Nitrophenol, morphine, acetaminophen, diazepam, *N*-hydroxydapsone, sulfathiazole, meprobamate, digitoxin, digoxin
Acetylation	Acetyl-CoA	*N*–Acetyltransferase (cytosol)	Amines	Sulfonamides, isoniazid, clonazepam, dapsone, mescaline
Glutathione conjugation	Glutathione (GSH)	GSH-*S*-transferase (cytosol, microsomes)	Epoxides, arene oxides, nitro groups, hydroxylamines	Acetaminophen, ethacrynic acid, bromobenzene
Glycine conjugation	Glycine	Acyl-CoA glycinetransferase (mitochondria)	Acyl-CoA derivatives of carboxylic acids	Salicylic acid, benzoic acid, nicotinic acid, cinnamic acid, cholic acid, deoxycholic acid
Sulfation	Phosphoadenosyl phosphosulfate	Sulfotransferase (cytosol)	Phenols, alcohols, aromatic amines	Estrone, aniline, phenol, 3-hydroxycoumarin, acetaminophen, methyldopa
Methylation	*S*-Adenosylmethionine	Transmethylases (cytosol)	Catecholamines, phenols, amines	Dopamine, epinephrine, pyridine, histamine, thiouracil
Water conjugation	Water	Epoxide hydrolase (microsomes) (cytosol)	Arene oxides, *cis*-disubstituted and monosubstituted oxiranes Alkene oxides, fatty acid epoxides	Benzopyrene 7,8-epoxide, styrene 1,2-oxide, carbamazepine epoxide Leukotriene A_4

Structures of cofactors for phase II biotransformation

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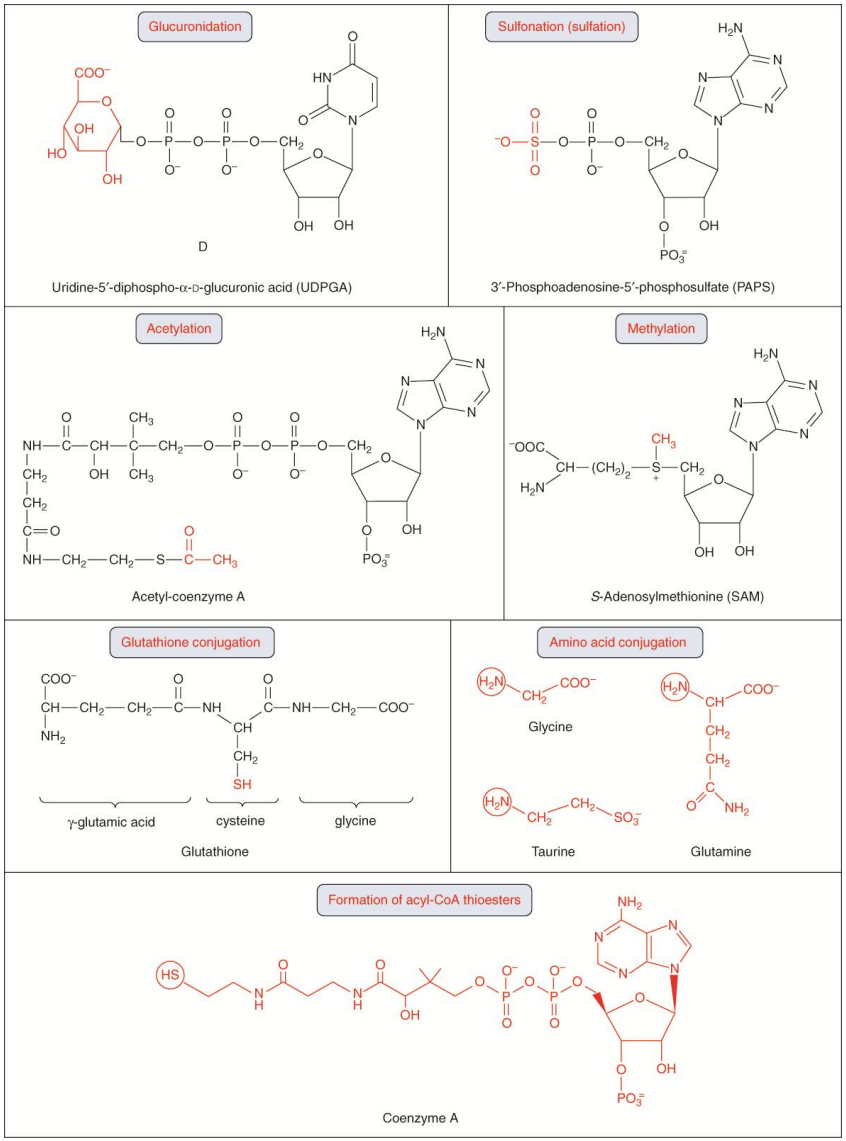

Factors influencing drug metabolism

What are these factors?

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GENETIC

Slow metabolizers
vs.
Fast metabolizers

PHYSIOLOGICAL

New-born show some enzymatic immaturity

Age decreases metabolic capacity

ENVIRONMENTAL

Way of life (alcohol, tobacco)

Food (grapefruit juice)

Drugs !!!

PATHOLOGICAL

Liver damages (NASH…etc.)

Inducibility of metabolism

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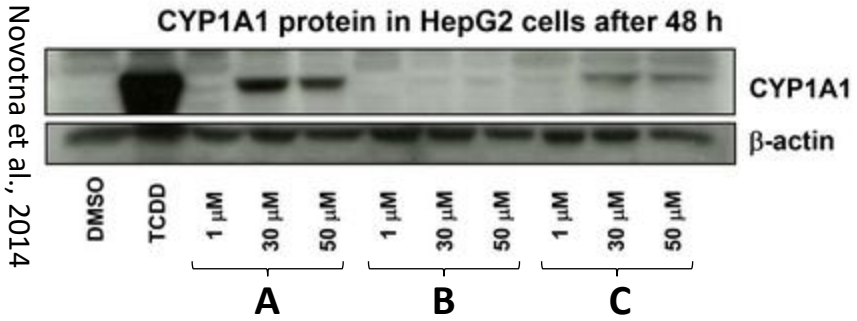
- ✓ Exposure to a xenobiotic is generally responsible for the **induction of an enzymatic system** capable of supporting this molecule;



- ✓ Response to toxicants is adaptative: cells and tissues will produce the enzymatic machinery to metabolize and eliminate a molecule only if this molecule has been detected;

- ✓ Although generally beneficial to the body, the metabolism of xenobiotics can sometimes paradoxically be the cause of **exacerbated toxicity by generating molecules or metabolites that are more toxic** than the native molecule.

Metabolic interactions : drugs examples

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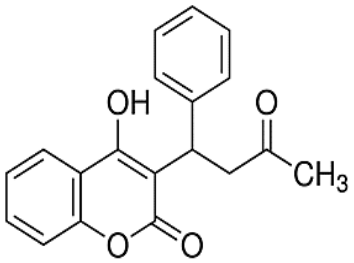
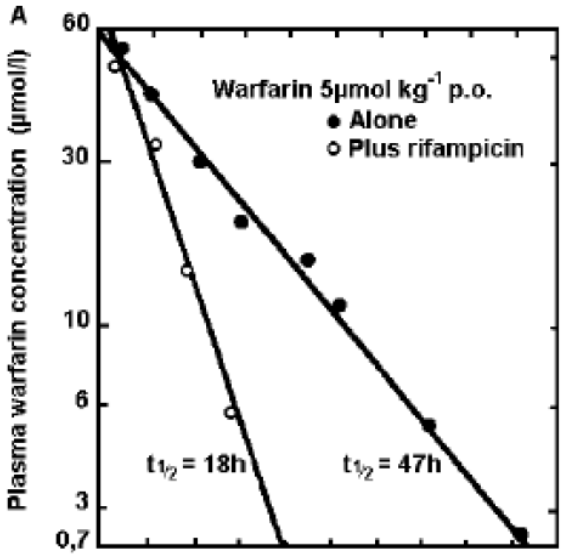
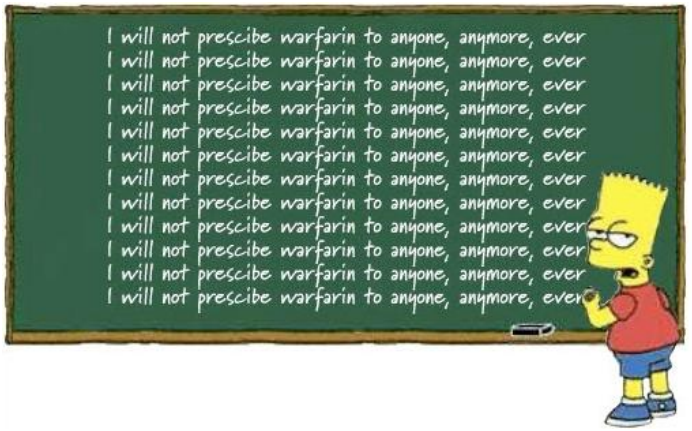
CYP2C9

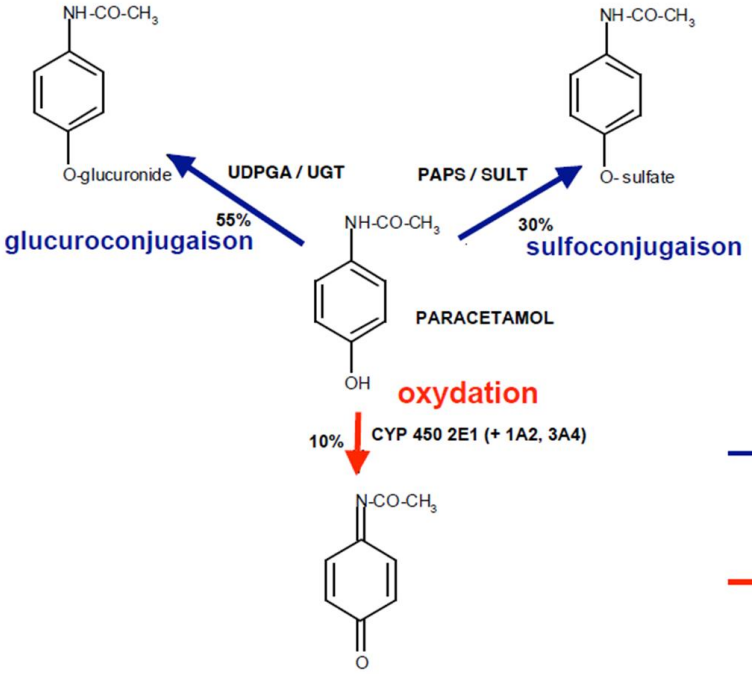


Warfarine

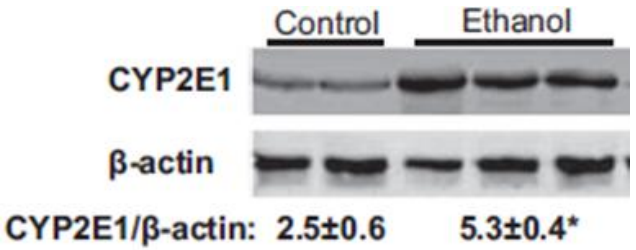




Acetaminophen/P4502E1

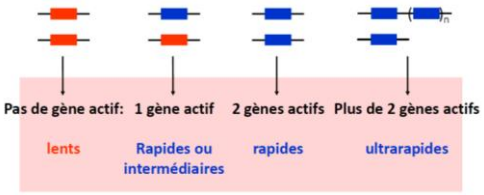


N-acétyl-p-benzoquinoneimine (NAPQ)

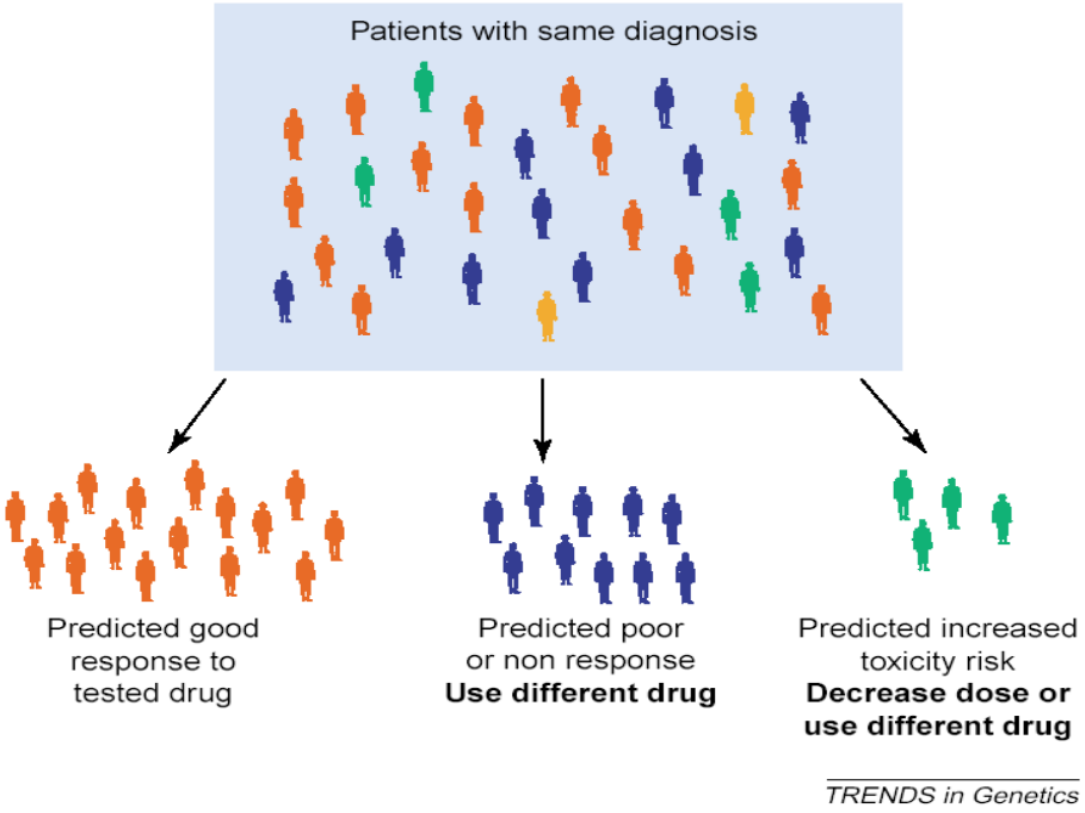


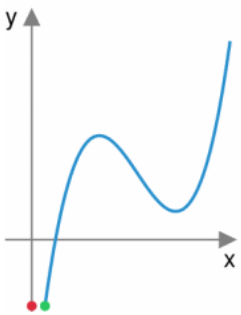
- 2 voies de détoxification : conjugaisons ➠ élimination
- 1 voie de toxification : CYP450 2E1 (+ 1A2, 3A4)

Consequences of CYP polymorphism



Molecular basis of CYP polymorphism





What therapeutic consequences?

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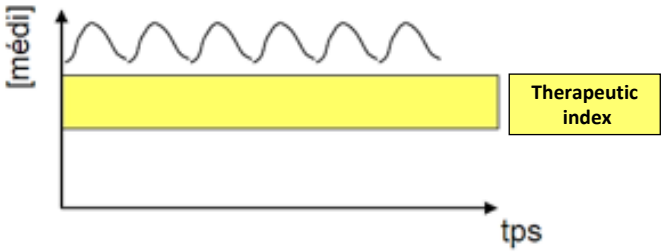
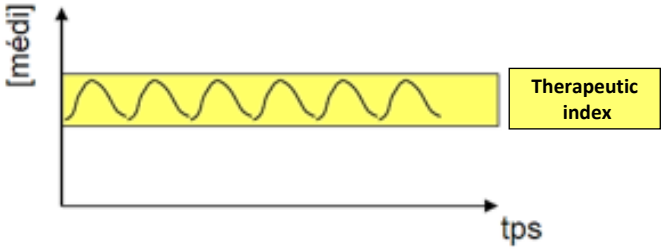
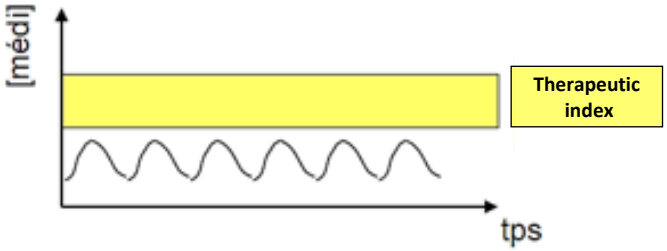
Phenotype	Effects on [drugs]	Clinical consequences: Active drugs	Clinical consequences: Prodrugs
Slow metabolizers		Toxicity	No therapeutic effects
Fast metabolizers		Therapeutic efficacy	
Ultrafast metabolizers		No therapeutic effects	Toxicity

What you need to know!

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Metabolism is one of the key elements of drug activity and toxicity

- 3 phases in general but the scheme can vary
- Effet de 1er passage hépatique
- Pro-drugs

Many enzymes are capable of metabolising drugs

- Major role of CYP450

These enzymes are subject to inhibition and induction = drug interactions

There is genetic polymorphism in the metabolizing enzymes

The toxicity of a molecule may exist after metabolization

- Bioactivation

Liste inducteurs/inhibiteurs P450/Pgp

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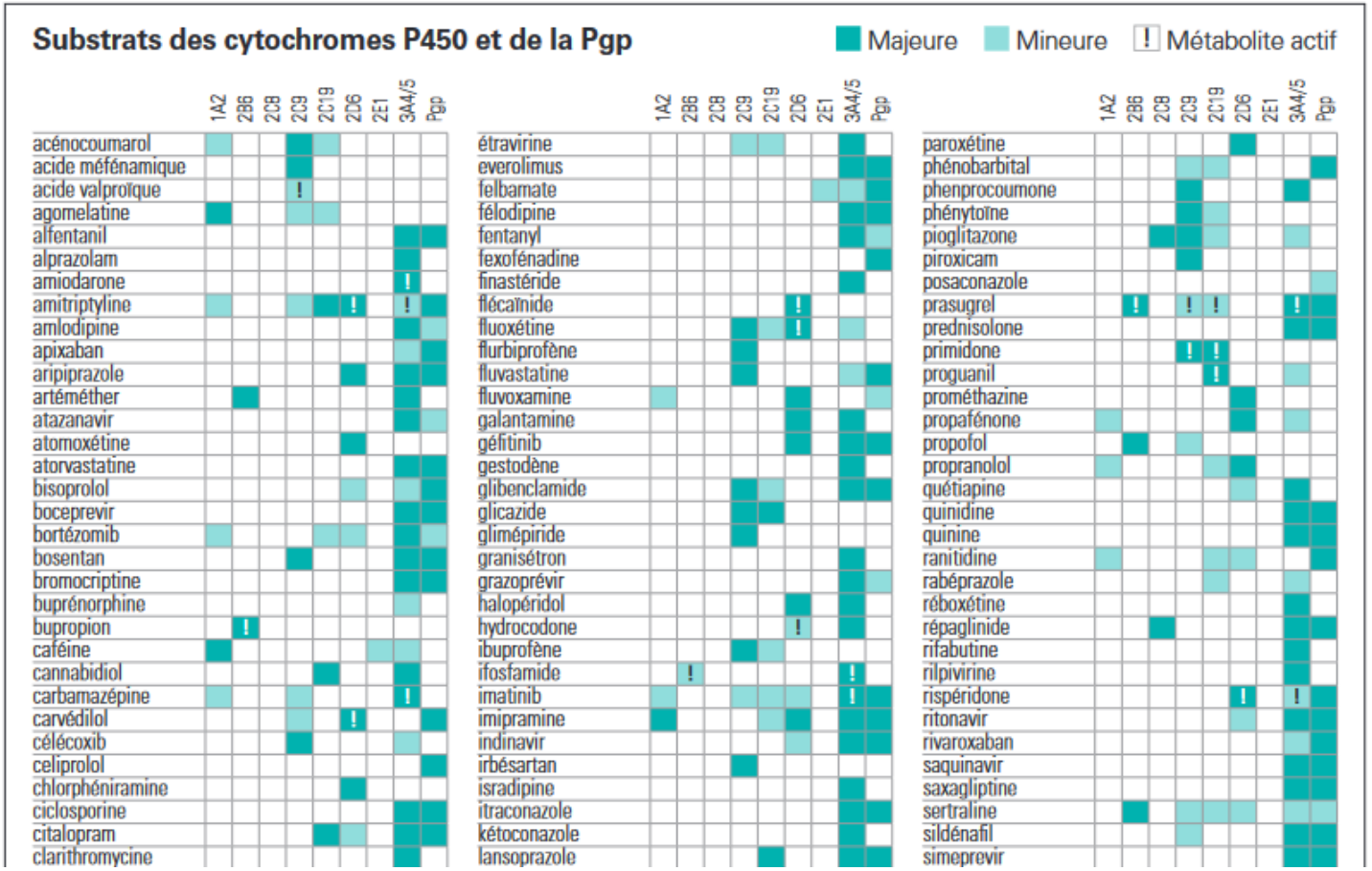
Document available on eCAMPUS

Interactions médicamenteuses, cytochromes P450 et P-glycoprotéine (Pgp)



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