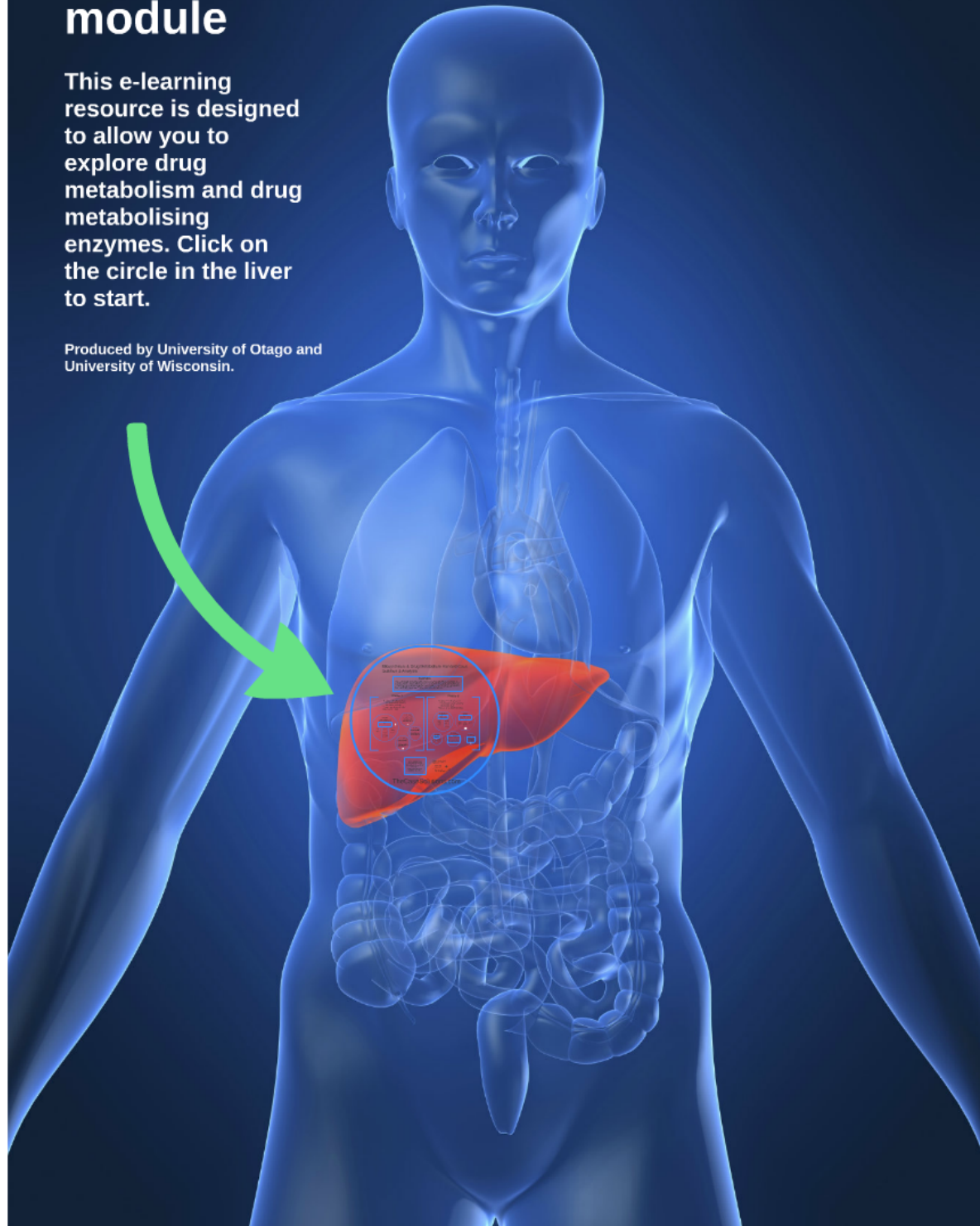


# Drug metabolism e-learning module

This e-learning resource is designed to allow you to explore drug metabolism and drug metabolising enzymes. Click on the circle in the liver to start.

Produced by University of Otago and University of Wisconsin.



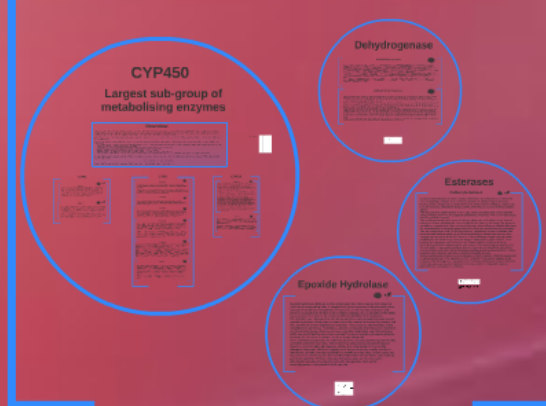
# Biosynthesis & Drug Metabolism Harvard Case Solution & Analysis

## Overview

In 1947 R. T. Williams noted that enzyme-catalyzed biotransformation of drugs, dietary substances, and other synthetic and environmental agents, i.e., xenobiotics, can be by synthetic and/or synthetic reactions. Enzymes catalyzing synthetic or so-called Phase 1, reactions are thus classified broadly as all those catalyzing the oxidation, reduction, and hydrolysis of xenobiotics. Enzymes in this group include, but are not limited to, the mixed-function oxidases more commonly known as cytochrome P450 monooxygenases, monooxygenases and dioxygenases, aldehyde dehydrogenases, aldehyde oxidase, alcohol dehydrogenases, quinone oxidoreductases, short-chain dehydrogenase/oxidoreductases, aldo-keto reductases, and esterases. Enzymes catalyzing synthetic, or so-called Phase 2, reactions are thus classified broadly as all those catalyzing the conjugation of xenobiotics to endogenous molecules, e.g., glucuronic acid, sulfate, glutathione, acetate, or a methyl group. Representative enzymes in this group include, but are not limited to, the UDP-glycosyltransferases, sulfotransferases, glutathione S-transferases, N-acetyltransferases, and methyltransferases.

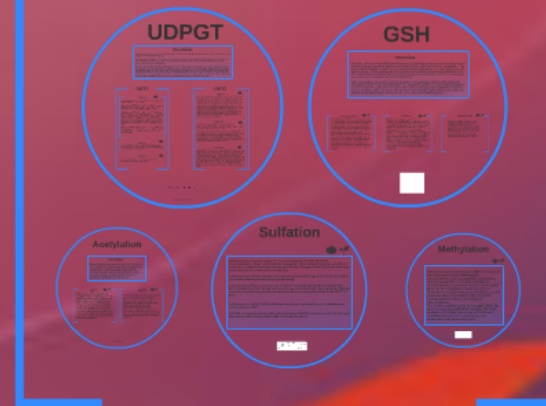
## Phase 1

Introduce a functional group  
 • Increases water solubility  
 • Increases reactivity  
 Product can be excreted or metabolised further.



## Phase 2

Conjugates a large group  
 • Increases water solubility  
 • Increases size  
 Product is normally excreted.



Drug metabolising enzymes are found throughout the body but the highest concentrations are found in the liver.

These symbols indicate whether the enzyme is relevant to:

- Clinical drug metabolism (green leaf icon)
- or
- Environmental chemical metabolism (blue leaf icon)

**Drug metabolising enzymes are found throughout the body but the highest concentrations are found in the liver.**

# Synthesis & Drug Metabolism Harvard Case Detection & Analysis

## Overview

In 1947 R. T. Williams noted that enzyme-catalyzed biotransformation of drugs, dietary substances, and other synthetic and environmental agents, i.e., xenobiotics, can be by asynthetic and/or synthetic reactions. Enzymes catalyzing asynthetic, or so-called Phase 1, reactions are thus classified broadly as all those catalyzing the oxidation, reduction, and hydrolysis of xenobiotics. Enzymes in this group include, but are not limited to, the mixed-function oxidases more commonly known as cytochrome P450 monooxygenases, monoamine and diamine oxidases, aldehyde dehydrogenases, aldehyde oxidase, alcohol dehydrogenases, quinone oxidoreductases, short-chain dehydrogenases/reductases, aldo-keto reductases, and esterases. Enzymes catalyzing synthetic, or so-called Phase 2, reactions are thus classified broadly as all those catalyzing the conjugation of xenobiotics to endogenous molecules, e.g., glucuronic acid, sulfate, glutathione, acetate, or a methyl group. Representative enzymes in this group include, but are not limited to, the UDP-glycosyltransferases, sulfotransferases, glutathione S-transferases, N-acetyltransferases, and methyltransferases

## Phase 1

Introduces a functional group  
Increases water solubility  
Increases reactivity  
Product can be excreted or  
undergo further.

## Phase 2

Conjugates a large group

- Increases water solubility
- Increases size

Product is normally excreted.

# Phase 1

Introduce a functional group

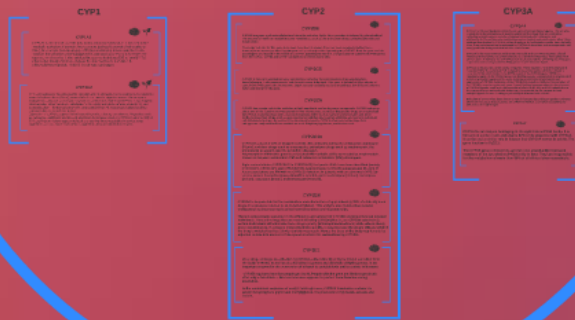
- Increases water solubility
- Increases reactivity

Produce can be excreted or metabolised further.

## CYP450 Largest sub-group of metabolising enzymes

### Overview

Cytochrome P450 (CYP) genes are responsible for metabolising drugs. They are a superfamily of enzymes, which are involved in the metabolism of a wide range of endogenous and exogenous compounds. The CYP450 superfamily is the largest and most diverse of the cytochrome P450 superfamily. It is composed of several subfamilies, each of which contains a number of different enzymes. The CYP450 superfamily is found in all eukaryotes, including plants, animals, and fungi. The CYP450 superfamily is involved in the metabolism of a wide range of compounds, including drugs, hormones, and neurotransmitters. The CYP450 superfamily is also involved in the synthesis of cholesterol and other lipids. The CYP450 superfamily is a large and diverse group of enzymes, and it is one of the most important groups of enzymes in the human body.



## Dehydrogenase

**Alcohol Dehydrogenase**  
Alcohol dehydrogenase (ADH) is a family of enzymes that catalyze the oxidation of alcohols to aldehydes or ketones. It is found in all eukaryotes and is one of the most abundant enzymes in the liver. ADH is involved in the metabolism of a wide range of drugs, including ethanol, acetaminophen, and aspirin. It is also involved in the synthesis of cholesterol and other lipids. ADH is a dimeric enzyme, meaning it is composed of two subunits. Each subunit contains a zinc atom and a heme prosthetic group. The zinc atom is coordinated to the N-terminal amino group of the enzyme, while the heme prosthetic group is coordinated to the C-terminal amino group. The heme prosthetic group is also coordinated to a cysteine residue in the active site of the enzyme. The zinc atom and the heme prosthetic group are both essential for the catalytic activity of ADH.



## Esterases

### Carboxylesterases

Carboxylesterases are a family of enzymes that catalyze the hydrolysis of esters, amides, and other compounds. They are found in all eukaryotes and are involved in the metabolism of a wide range of drugs, including aspirin, acetaminophen, and ibuprofen. Carboxylesterases are also involved in the synthesis of cholesterol and other lipids. There are several different types of carboxylesterases, each with a different substrate specificity. The most common type is carboxylesterase 1 (CE1), which is found in the liver and is involved in the metabolism of a wide range of drugs. CE1 is a dimeric enzyme, meaning it is composed of two subunits. Each subunit contains a zinc atom and a heme prosthetic group. The zinc atom is coordinated to the N-terminal amino group of the enzyme, while the heme prosthetic group is coordinated to the C-terminal amino group. The heme prosthetic group is also coordinated to a cysteine residue in the active site of the enzyme. The zinc atom and the heme prosthetic group are both essential for the catalytic activity of CE1.



## Epoxide Hydrolase

Epoxide hydrolases (EH) are a class of enzymes that cleave oxirane derivatives to yield the corresponding diols. It is named from the perspective of the classical phase concept of metabolic transformation, but had a life at the interface of Phase I and Phase II, as epoxide hydrolase may as well be regarded as a conjugation with water because water is added to the molecule without splitting it into fragments. The first EH to be characterized was the membrane-bound mammalian microsomal epoxide hydrolase which plays a major role in the control of chemically reactive and thus potentially cytotoxic epoxide intermediates. These arise as intermediates in the metabolism of numerous xenobiotics, and their metabolic detoxification is therefore of primary importance. A few years later a soluble mammalian epoxide hydrolase (sEH) was identified that was able to convert the neurotoxic molecule piperonyl butoxide (PB) into a diol, an oxiran derivative, to the corresponding diol. In the mammalian organism, the sEH has shown to be particularly important for the control of genotoxic epoxides. The enzyme has broad substrate specificity and displays a surprisingly high apparent affinity to a wide variety of structurally divergent substrates, while the hydrolysis of these substrates usually results in formation of their chemical reactivity, the resulting metabolites, in few cases, are the precursors of third-generation reactive metabolites of nonreactive ordinary genotoxic potential. Famous examples for such a case are the bay region dihydrodiol epoxides of polycyclic aromatic hydrocarbons, such as the benzo[a]pyrene 7,8-epoxide, 9,10-epoxide.



# CYP450

## Largest sub-group of metabolising enzymes

### Overview

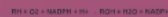
Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a heme co-factor and, therefore, are haemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, usually organised as P450-containing systems. The heme P450 is derived from the cytochrome peak at the N-terminus of the absorption maximum of the enzyme (450 nm) where it is the reduced state of cytochrome b5.

CYP enzymes have been identified in all domains of life - animals, plants, fungi, protists, bacteria, archaea, and even in viruses. However, the enzymes have not been found in *E. coli*. More than 2,000 distinct CYP proteins are known.

Most CYPs require a protein partner to deliver one or more electrons to reduce the heme (and eventually molecular oxygen). Based on the nature of the electron transfer proteins, CYPs can be classified into several groups:

- Microsomal P450 systems in which electrons are transferred from NADPH via cytochrome P450 reductase (usually CYP<sub>1</sub>, PDR, or CYPOR). Cytochrome b5 (b5) can also contribute reducing power to this system, after being reduced by cytochrome b5 reductase (C70R).
- Mitochondrial P450 systems, that employ adrenodoxin-reduced and adrenodoxin-reduced iron-sulfur (AdoR) as P450.
- Mitochondrial P450 systems, that employ a ferredoxin reductase and ferredoxin by ferredoxin reductase (FDR).
- CYP119-AdoR systems in which both electrons required for the CYP come from cytochrome b5.
- P450-NADPH systems employed by bacteria in which a P450 domain and heme reductase is fused to the CYP.
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- P450-NADPH systems employed by bacteria in which a P450 domain and heme reductase is fused to the CYP.

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the alpha position of an organic substrate (R) while the other oxygen atom is reduced to water:



Humans have 57 genes and more than 18 pseudogenes divided among 18 families of cytochrome P450 genes and 43 subfamilies.

Table 1. CYP Gene Families and Subfamilies

Family	Subfamily	Gene	Location	Function
CYP1	CYP1A1	CYP1A1	Chromosome 2q37	Phase I metabolism of polycyclic aromatic hydrocarbons (PAHs)
		CYP1A2	Chromosome 10q24	Phase I metabolism of PAHs and other xenobiotics
CYP2	CYP2B6	CYP2B6	Chromosome 19p13.3	Phase I metabolism of drugs like propofol and etomidate
		CYP2C8	Chromosome 10q24	Phase I metabolism of drugs like paclitaxel and docetaxel
		CYP2C9	Chromosome 10q24	Phase I metabolism of drugs like warfarin and phenytoin
CYP3A	CYP3A4	CYP3A4	Chromosome 12p21	Phase I metabolism of drugs like midazolam and triazolam
		CYP3A5	Chromosome 12p21	Phase I metabolism of drugs like midazolam and triazolam
		CYP3A7	Chromosome 12p21	Fetal phase I metabolism of drugs like midazolam and triazolam

### CYP1

**CYP1A1** is also known as liver phase I metabolism. It is found in the placenta and various tissues. It is involved in the metabolism of polycyclic aromatic hydrocarbons (PAHs), for example, and polycyclic aromatic hydrocarbons (PAHs). The primary site of metabolism is located in the CYP1A1 gene on 2q37. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP1A1 variants are known to be polymorphic.

**CYP1A2** functions in the endoplasmic reticulum and its expression is regulated by various factors. It is involved in the metabolism of PAHs and other xenobiotics. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP1A2 variants are known to be polymorphic.

### CYP2

**CYP2B6** converts cytochrome b5-reduced iron-sulfur (AdoR) to cytochrome b5-reduced iron-sulfur (AdoR). It is involved in the metabolism of drugs like propofol and etomidate. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP2B6 variants are known to be polymorphic.

**CYP2C8** is a member of the cytochrome P450 superfamily. It is involved in the metabolism of drugs like paclitaxel and docetaxel. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP2C8 variants are known to be polymorphic.

**CYP2C9** is a member of the cytochrome P450 superfamily. It is involved in the metabolism of drugs like warfarin and phenytoin. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP2C9 variants are known to be polymorphic.

**CYP2C19** acts on 50% of drugs in phase I metabolism. It is involved in the metabolism of drugs like omeprazole and esomeprazole. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP2C19 variants are known to be polymorphic.

**CYP2D6** is responsible for the metabolism and elimination of approximately 25% of clinically used drugs. It is involved in the metabolism of drugs like debrisoquine and dextropropripramine. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP2D6 variants are known to be polymorphic.

**CYP2E1** is the major enzyme for the metabolism of alcohol. It is involved in the metabolism of drugs like ethanol and acetaldehyde. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP2E1 variants are known to be polymorphic.

### CYP3A

**CYP3A4** is induced by glucocorticoids and other steroid hormones. It is involved in the metabolism of drugs like midazolam and triazolam. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP3A4 variants are known to be polymorphic.

**CYP3A5** is a member of the cytochrome P450 superfamily. It is involved in the metabolism of drugs like midazolam and triazolam. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP3A5 variants are known to be polymorphic.

**CYP3A7** is an enzyme belonging to the cytochrome P450 family. It is involved in the metabolism of drugs like midazolam and triazolam. It is highly inducible by specific hydrocarbons such as benzo(a)pyrene. Many CYP3A7 variants are known to be polymorphic.

## Epoxide Hy

Epoxide hydrolases (EH) are a class of enzymes that catalyze the hydrolysis of epoxides to yield the corresponding diols. If viewed from the perspective of xenobiotic metabolism, EH lead a life in phase II, as epoxide hydrolysis may as well be regarded as a phase II reaction because water is added to the molecule without the loss of any functional groups. The first EH to be characterized was the membrane-bound EH from sheep liver.

Alcohol dehydrogenase (ADH) is a class of enzymes that catalyze the oxidation of alcohols to aldehydes or ketones. The first ADH to be characterized was the membrane-bound ADH from sheep liver.

Aldehyde oxidase (AO) is a class of enzymes that catalyze the oxidation of aldehydes to carboxylic acids. The first AO to be characterized was the membrane-bound AO from sheep liver.

# Largest sub-group of metabolising enzymes

## Overview

Cytochromes P450 (CYPs) belong to the superfamily of proteins containing a haem co-factor and, therefore, are haemoproteins. CYPs use a variety of small and large molecules as substrates in enzymatic reactions. They are, in general, the terminal oxidase enzymes in electron transfer chains, broadly categorised as P450-containing systems. The term P450 is derived from the spectrophotometric peak at the wavelength of the absorption maximum of the enzyme (450 nm) when it is in the reduced state and complexed with carbon monoxide.

CYP enzymes have been identified in all domains of life - animals, plants, fungi, protists, bacteria, archaea, and even in viruses. However, the enzymes have not been found in *E. coli*. More than 21,000 distinct CYP proteins are known.

Most CYPs require a protein partner to deliver one or more electrons to reduce the iron (and eventually molecular oxygen). Based on the nature of the electron transfer proteins, CYPs can be classified into several groups:

- Microsomal P450 systems in which electrons are transferred from NADPH via cytochrome P450 reductase (variously CPR, POR, or CYPOR). Cytochrome b5 (cyb5) can also contribute reducing power to this system after being reduced by cytochrome b5 reductase (CYB5R).
- Mitochondrial P450 systems, that employ adrenodoxin reductase and adrenodoxin to transfer electrons from NADPH to P450.
- Bacterial P450 systems, that employ a ferredoxin reductase and a ferredoxin to transfer electrons to P450.
- CYB5R/cyb5/P450 systems in which both electrons required by the CYP come from cytochrome b5.
- FMN/Fd/P450 systems originally found in *Rhodococcus* sp. in which a FMN-domain-containing reductase is fused to the CYP.
- P450 only systems, which do not require external reducing power. Notable ones include CYP5 (thromboxane synthase), CYP8 (prostacyclin synthase), and CYP74A (allene oxide synthase).

The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction, e.g., insertion of one atom of oxygen into the aliphatic position of an organic substrate (RH) while the other oxygen atom is reduced to water:



Humans have 57 genes and more than 59 pseudogenes divided among 18 families of cytochrome P450 genes and 43 subfamilies. [

## CYP2

### CYP2B6

CYP2B6 enzymes synthesise cholesterol, steroids and other lipids. Its expression is induced by phenobarbital. The enzyme is known to metabolize some xenobiotics, such as the anti-cancer drugs cyclophosphamide and ifosfamide.

Transcript variants for this gene have been described; however, it has not been resolved whether these transcripts are in fact produced by this gene or by a closely related pseudogene, CYP2B7. Both the gene and the pseudogene are located in the middle of a CYP2A pseudogene found in a large cluster of cytochrome P450 genes from the CYP2A, CYP2B and CYP2F subfamilies on chromosome 19q.

CYP3A4 is induced by glucocorticoids. It is involved in the metabolism of many drugs, including acetaminophen, erythromycin. The enzyme undergoes deactivation by CYP3A4. Also, many substances are many protoxins being toxic.

CYP3A4 is the most common member of this family, it is an iron atom. In humans, it is part of a cluster of cytochromes.



...ylase). It is involved in the metabolism of many drugs, including acetaminophen, erythromycin. The enzyme undergoes deactivation by CYP3A4. Also, many substances are many protoxins being toxic.

# Table of CYP450 isoforms

Family	Function	Members	Names
CYP1	drug and steroid (especially estrogen) metabolism, benzo(a)pyrene toxification	3 subfamilies, 3 genes, 1 pseudogene	CYP1A1, CYP1A2, CYP1B1
CYP2	drug and steroid metabolism	13 subfamilies, 16 genes, 16 pseudogenes	CYP2A6, CYP2A7, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1, CYP2S1, CYP2U1, CYP2W1
CYP3	drug and steroid (including testosterone) metabolism	1 subfamily, 4 genes, 2 pseudogenes	CYP3A4, CYP3A5, CYP3A7, CYP3A43
CYP4	arachidonic acid or fatty acid metabolism	6 subfamilies, 12 genes, 10 pseudogenes	CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP4F3, CYP4F8, CYP4F11, CYP4F12, CYP4F22, CYP4V2, CYP4X1, CYP4Z1
CYP5	thromboxane A2 synthase	1 subfamily, 1 gene	CYP5A1
CYP7	bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus	2 subfamilies, 2 genes	CYP7A1, CYP7B1
CYP8	varied	2 subfamilies, 2 genes	CYP8A1 (prostacyclin synthase), CYP8B1 (bile acid biosynthesis)
CYP11	steroid biosynthesis	2 subfamilies, 3 genes	CYP11A1, CYP11B1, CYP11B2
CYP17	steroid biosynthesis, 17-alpha hydroxylase	1 subfamily, 1 gene	CYP17A1
CYP19	steroid biosynthesis: aromatase synthesizes estrogen	1 subfamily, 1 gene	CYP19A1
CYP20	unknown function	1 subfamily, 1 gene	CYP20A1
CYP21	steroid biosynthesis	2 subfamilies, 1 gene, 1 pseudogene	CYP21A2
CYP24	vitamin D degradation	1 subfamily, 1 gene	CYP24A1
CYP26	retinoic acid hydroxylase	3 subfamilies, 3 genes	CYP26A1, CYP26B1, CYP26C1
CYP27	varied	3 subfamilies, 3 genes	CYP27A1 (bile acid biosynthesis), CYP27B1 (vitamin D3 1-alpha hydroxylase, activates vitamin D3), CYP27C1 (unknown function)
CYP39	7-alpha hydroxylation of 24-hydroxycholesterol	1 subfamily, 1 gene	CYP39A1
CYP46	cholesterol 24-hydroxylase	1 subfamily, 1 gene	CYP46A1
CYP51	cholesterol biosynthesis	1 subfamily, 1 gene, 3 pseudogenes	CYP51A1 (lanosterol 14-alpha demethylase)