

# Cytochrome P450 Polymorphisms and Drug Metabolism

## Pharmacogenomics Course Spring 2008

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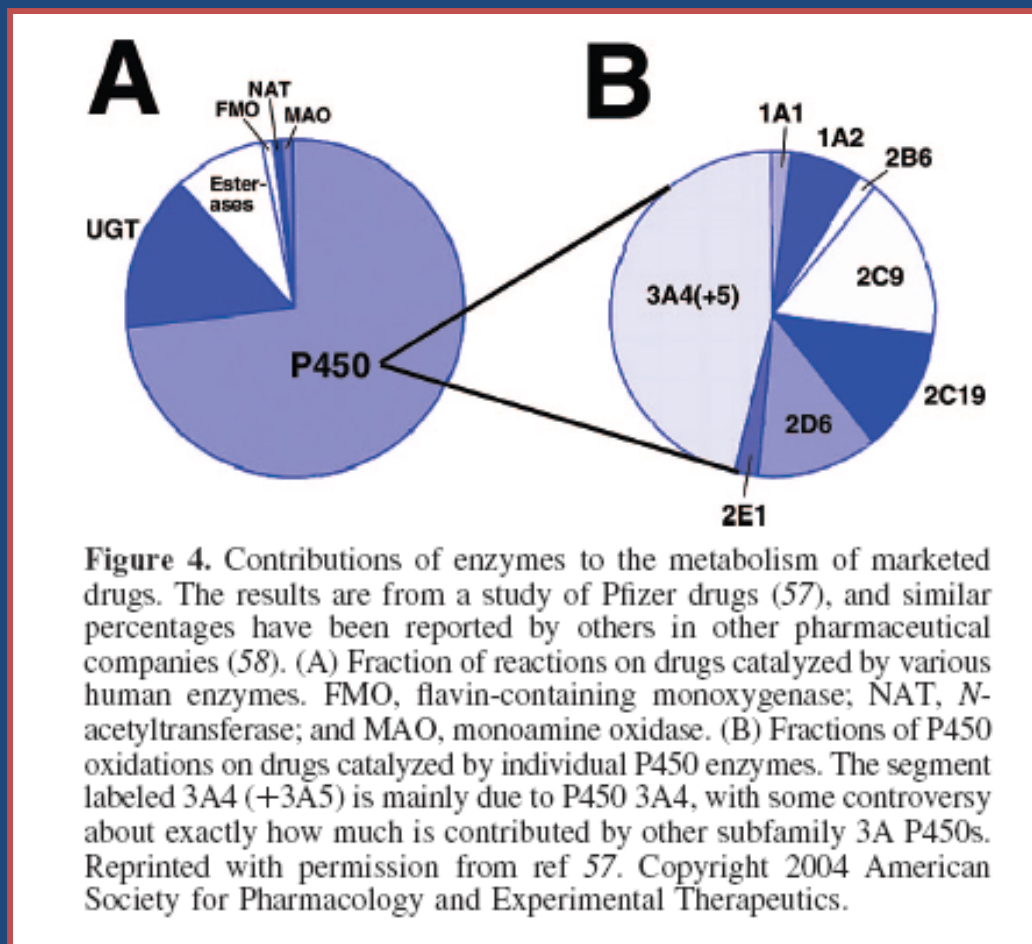
# Overview

Different CYPs involved in drug metabolism

Mechanisms of CYP action

Effects of mutations and polymorphisms in CYPs on drug metabolism

# P450s are the major enzymes involved in drug metabolism



Accounting for ~75%  
of drug metabolism

# P450s are classified on basis of substrates

**Table 1. Classification of Human Cytochrome P450s Based on Major Substrate Class<sup>2,3</sup>**

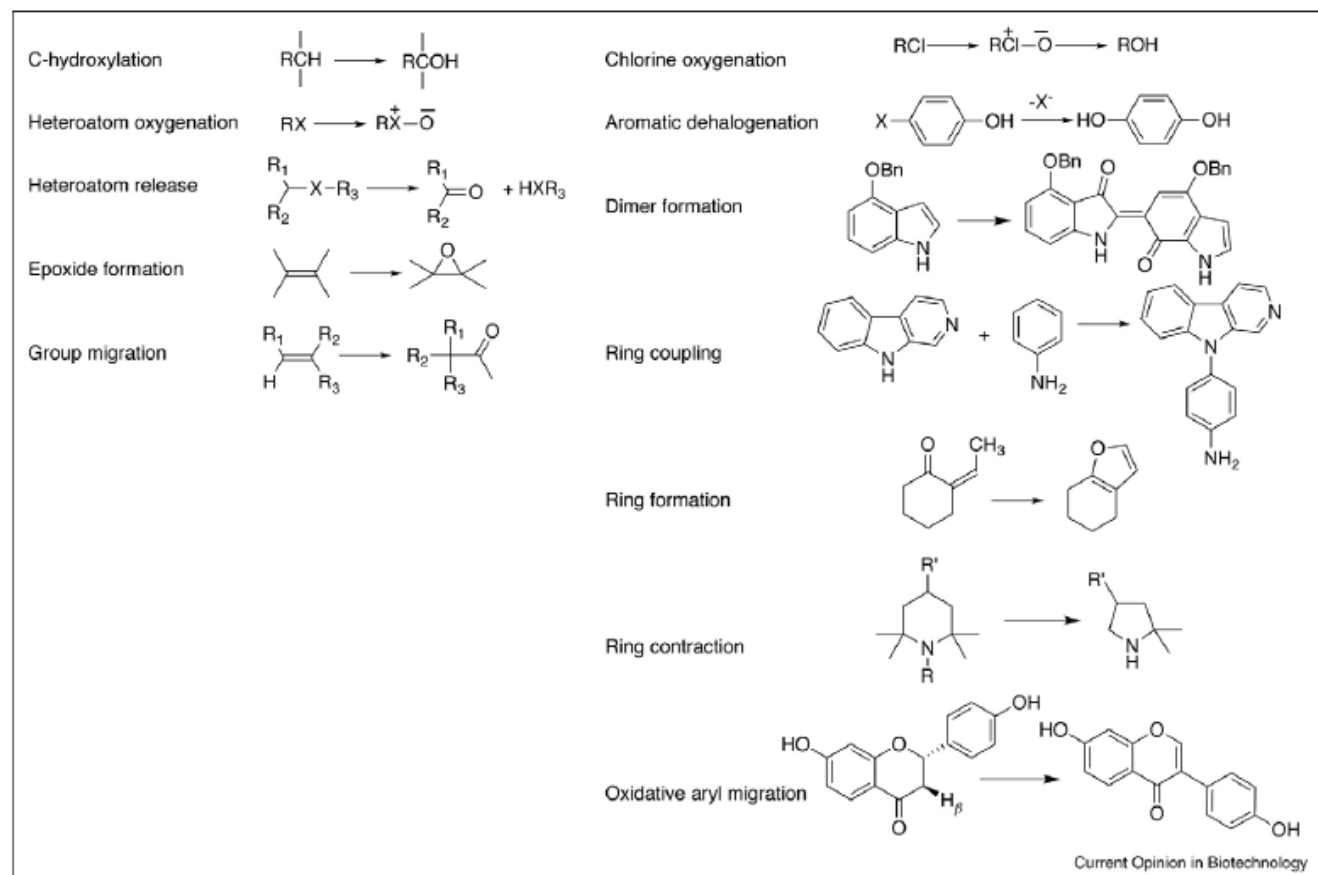
	<b>Fatty</b>				
<b>Sterols</b>	<b>Xenobiotics</b>	<b>Acids</b>	<b>Eicosanoids</b>	<b>Vitamins</b>	<b>Unknown</b>
1B1	1A1	2J2	4F2	2R1	2A7
7A1	1A2	4A11	4F3	24A1	2S1
7B1	2A6	4B1	4F8	26A1	2U1
8B1	2A13	4F12	5A1	26B1	2W1
11A1	2B6		8A1	26C1	3A43
11B1	2C8			27B1	4A22
11B2	2C9				4F11
17A1	2C18				4F22
19A1	2C19				4V2
21A2	2D6				4X1
27A1	2E1				4Z1
39A1	2F1				20A1
46A1	3A4				27C1
51A1	3A5				
	3A7				

# Major human liver cytochrome P450 (CYP) enzymes

CYP	Drug substrate	Marker substrate/ reaction	Inhibitor	Inducer
1A2	Paracetamol (acetaminophen), caffeine, ondansetron, phenacetin, tacrine, tamoxifen, theophylline	Phenacetin O-de-ethylation	Furafylline	Smoking, charred food
2A6	Coumarin, nicotine	Coumarin 7-hydroxylation	Ditiocarb sodium (diethyldithio- carbamate)	
2C9	Diclofenac, flurbiprofen, losartan, phenytoin, piroxicam, tienilic acid, tolbutamide, torasemide, (S)-warfarin	Tolbutamide methyl hydroxylation	Sulfaphenazole	Barbiturates, rifampicin (rifampin)
2C19	Diazepam, (S)-mephenytoin, omeprazole, pentamidine, propranolol, (R)-warfarin	(S)-mephenytoin 4'-hydroxylation		
2D6	Bufuralol, codeine, debrisoquine, desipramine, dextromethorphan, encainide, fluoxetine, haloperidol, imipramine, nortriptyline, paroxetine, propafenone, propranolol, sparteine	Bufuralol 1'-hydroxylation	Quinidine, ajmaline	
2E1	Paracetamol, caffeine, chlorzoxazone, enflurane, theophylline	Chlorzoxazone 6-hydroxylation	Ditiocarb sodium	Alcohol (ethanol), isoniazid
3A4	Benzphetamine, clarithromycin, codeine, cyclosporin, dapsone, diazepam, erythromycin, felodipine, tacrolimus, indinavir, lovastatin, midazolam, nifedipine, carbamazepine, losartan, quinidine, taxol, terfenadine, verapamil	Testosterone 6 $\beta$ -hydroxylation	Gestodene, troleandomycin, L-754,394, ketoconazole, itraconazole	Barbiturates, rifampicin, dexamethasone, carbamazepine

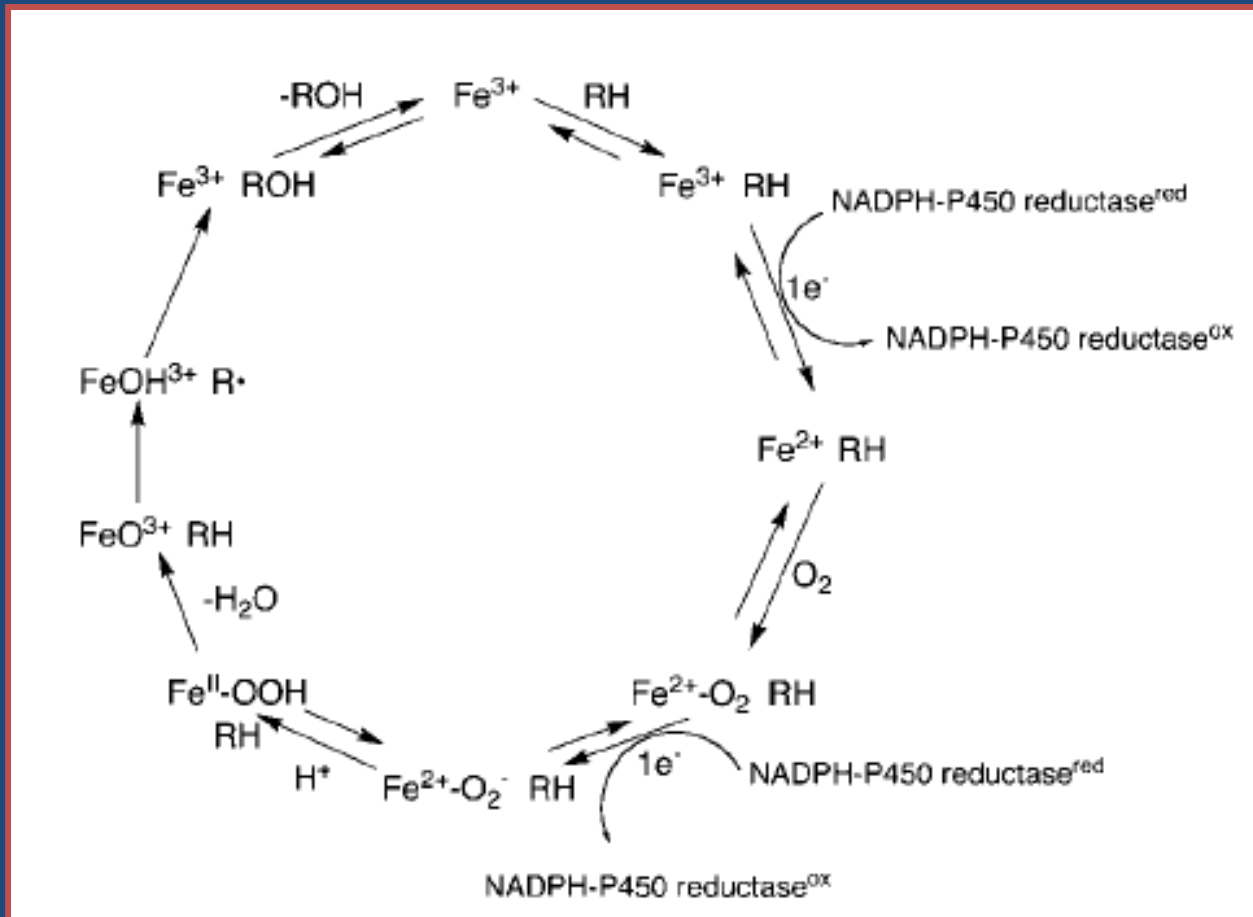
# CYP enzymatic reactions

Figure 1

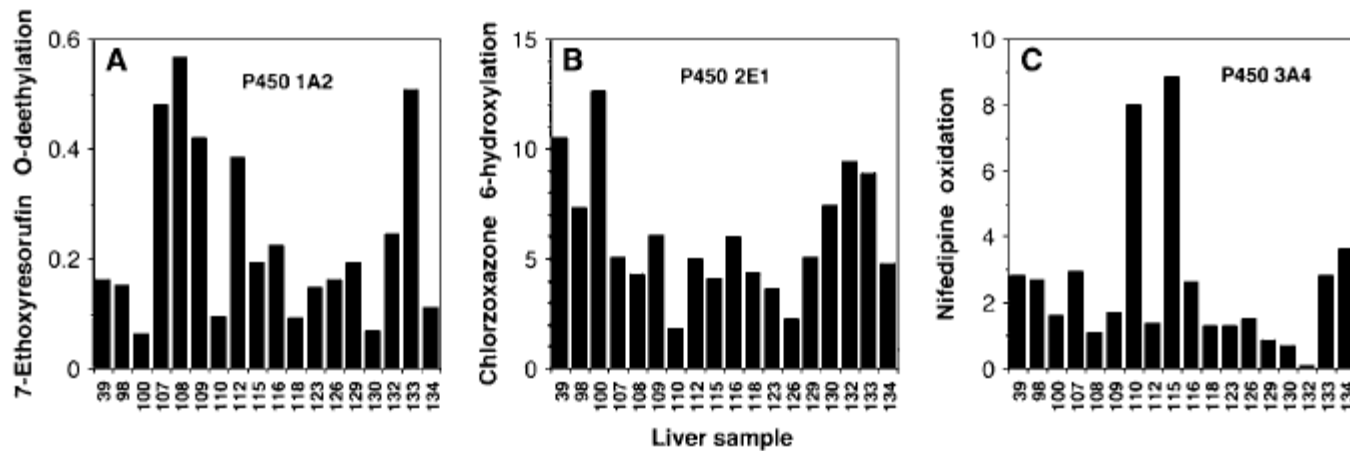


Examples of chemical reactions catalysed by CYP enzymes. Many are formal oxidations, but reductions and rearrangements are also known.

# Catalytic cycle for P450 reactions

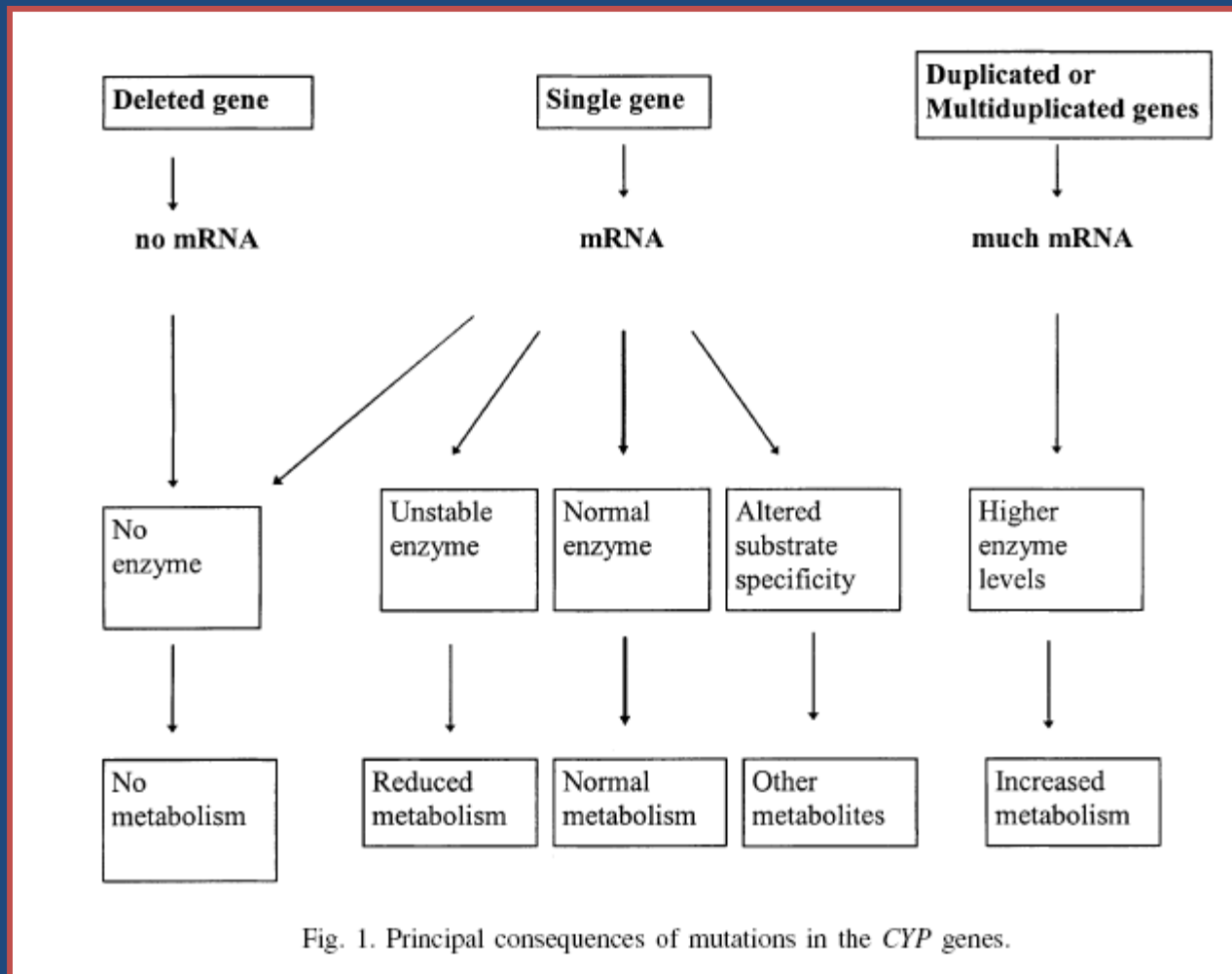


# Variability in levels of the “xenobiotic-metabolizing” CYPs (P450s)



**Figure 2.** Variability in levels of individual P450s in 18 human liver samples (designated with code numbers). Marker activities are used for each P450; immunochemical assays yield similar patterns. P450 indicates cytochrome P450.<sup>2</sup>

# Effects of mutations in the *CYP* genes



Can cause enzyme products with abolished, reduced, altered or increased enzyme activity.

**Table 1** Human cytochrome P450 genes and their polymorphisms. The table is an update of the human P450 genes identified in July 2002 and the data regarding the gene names and localisation are from (<http://dmelson.utmem.edu/CytochromeP450.html>).

PG denotes pseudogene (in *italic*) and MP denotes that missense mutations have been described. Those marked *MP* in *italic* are present on the Human CYP allele nomenclature Committee web page (<http://www.imm.ki.se/CYPalleles/>). *nd* not described

CYP gene	Chromosome	Polymorphism missense (MP)/pseudogene (PG)	In vivo importance of the polymorphism <sup>a</sup>	CYP gene	Chromosome	Polymorphism missense MP/pseudogene PG	In vivo importance of the polymorphism <sup>a</sup>
<i>1A1</i>	15	<i>MP</i>		<i>4F3</i>	19	nd	
<i>1A2</i>	15	<i>MP</i>	Yes <b>Carcinogen</b>	<i>4F8</i>	19	nd	
<i>1B1</i>	2	<i>MP</i>	Perhaps	<i>4F9P</i>	19	<i>PG</i>	
<i>1P</i>	9	<i>PG</i>		<i>4F10P</i>	19	<i>PG</i>	
<i>2A6</i>	19	<i>MP</i>	Yes <b>Nicotine</b>	<i>4F11</i>	19	nd	
<i>2A7</i>	19	<i>PG?</i>		<i>4F12</i>	19	nd	
<i>2A7PT</i>	19	<i>PG</i>		<i>4F22</i>	19	nd	
<i>2A7PC</i>	19	<i>PG</i>		<i>4F23P</i>	19	<i>PG</i>	
<i>2A13</i>	19	nd		<i>4F24P</i>	19	<i>PG</i>	
<i>2A18P</i>	19	<i>PG</i>		<i>4F25P</i>	15	<i>PG</i>	
<i>2B6</i>	19	<i>MP</i>	Perhaps <b>Barbits</b>	<i>4F26P</i>	9	<i>PG</i>	
<i>2B7P1</i>	19	<i>PG</i>		<i>4F27P</i>	2	<i>PG</i>	
<i>2B7P2</i>	19	<i>PG</i>		<i>4F28</i>	21	nd	
<i>2B7P3</i>	19	<i>PG</i>		<i>4V2</i>	4	nd	
<i>2C8</i>	10	<i>MP</i>	Yes	<i>4X1</i>	1	nd	
<i>2C9</i>	10	<i>MP</i>	Yes	<i>5A1</i>	7	<i>MP</i>	Yes <b>PGs, LKs</b>
<i>2C18</i>	10	<i>MP</i>		<i>7A1</i>	8	nd	
<i>2C19</i>	10	<i>MP</i>	Yes <b>Drugs</b>	<i>7B1</i>	8	nd	
<i>2CP</i>	10	<i>PG</i>		<i>8A1</i>	20	<i>MP</i>	Yes <b>PGs, LKs</b>
<i>2D6</i>	22	<i>MP</i>	Yes	<i>8B1</i>	3	nd	
<i>2D7P</i>	22	<i>PG</i>		<i>11A1</i>	8?	nd	
<i>2D8P</i>	22	<i>PG</i>		<i>11B1</i>	8	nd	
<i>2E1</i>	10	<i>MP</i>		<i>11B2</i>		nd	
<i>2F1</i>	19	<i>MP?</i>		<i>17</i>	10	<i>MP</i>	Yes <b>Sterols</b>
<i>2F1P</i>	19	<i>PG</i>		<i>19</i>	15	<i>MP</i>	Perhaps
<i>2G1P</i>	19	<i>PG</i>		<i>21A2</i>	6	<i>MP</i>	Yes <b>Sterols</b>
<i>2G2P</i>	19	<i>PG</i>		<i>24</i>	20	nd	
<i>2J2</i>	8	<i>MP</i>		<i>26A1</i>	10	nd	
<i>2R1</i>	11	nd		<i>26B1</i>	2	nd	
<i>2S1</i>	19	nd		<i>26C1</i>	10	nd	
<i>2T2P</i>	19	<i>PG</i>		<i>27A1</i>	2	<i>MP</i>	Yes <b>Sterols</b>
<i>2T3P</i>	19	<i>PG</i>		<i>27B1</i>	2	nd	
<i>2U1</i>	4	nd		<i>27C1</i>	2	nd	
<i>2W1</i>	7	nd		<i>39A1</i>	6	nd	
<i>3A4</i>	7	<i>MP</i>		<i>46</i>	1	nd	
<i>3A5</i>	7	<i>MP</i>	Perhaps	<i>46P</i>	1	<i>PG</i>	
<i>3A5P1</i>	7	<i>PG</i>		<i>51</i>	7	nd	
<i>3A5P2</i>	7	<i>PG</i>		<i>51P1</i>	3	<i>PG</i>	
<i>3A7</i>	7	<i>MP</i>		<i>51P2</i>	13	<i>PG</i>	
<i>3A43</i>	7	nd	Functional?				
<i>4A11</i>	1	nd					
<i>4A20</i>	1	nd					
<i>4A20P</i>	1	<i>PG</i>					
<i>4B1</i>	1	nd					
<i>4F2</i>	19	nd					

<sup>a</sup>This column denotes polymorphism of importance for the in vivo function of the enzyme in question

# Polymorphisms in the human cytochrome P450 (CYP) genes

Difference in characteristics of genes encoding CYPs responsible for xenobiotics metabolism (CYP1-3) and those important for the metabolism of endogenous compounds.

Barbits = barbituates

PGs = prostaglandins

LKs = leukotrienes

Ingelman-Sundberg M. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol.* 2004 Jan;369(1):89-104.

# Properties and polymorphisms of major CYP forms important in human drug metabolism

**Table 1. Relative importance of polymorphisms in human cytochrome P450 enzymes involved in drug metabolism**

Enzyme	Fraction of drug metabolism (%) <sup>a</sup>	Substrates	Major allelic variants <sup>b</sup>	Clinical effects of the polymorphism	Significance of the polymorphism <sup>c</sup>
CYP1A2	5	Drugs, carcinogens	<i>CYP1A2*1K</i>	Less enzyme expression and inducibility	+
CYP2A6	2	Nicotine, drugs, carcinogens	<i>CYP2A6*4</i> , <i>CYP2A6*9</i>	Altered nicotine metabolism	+
CYP2B6	2–4	Drugs	–	Significant for the metabolism of cancer drugs	+
CYP2C8	1	Drugs	<i>CYP2C8*3</i>	Altered taxol metabolism	+
CYP2C9	10	Drugs	<i>CYP2C9*2</i> , <i>CYP2C9*3</i>	Drug dosage <sup>d</sup>	+++
CYP2C19	5	Drugs	<i>CYP2C19*2</i> , <i>CYP2C19*3</i>	Drug dosage <sup>d</sup> , drug efficacy	+++
CYP2D6	20–30	Drugs	<i>CYP2D6*2xm</i>	No response	+++
			<i>CYP2D6*4</i>	Drug dosage <sup>d</sup>	+++
			<i>CYP2D6*10</i>	Drug dosage <sup>d</sup>	+
			<i>CYP2D6*17</i>	Drug dosage <sup>d?</sup>	+
			<i>CYP2D6*41</i>	Drug dosage <sup>d?</sup>	+
CYP2E1	2–4	Carcinogens, solvents, drugs	–	No conclusive studies	–
CYP3A4	40–45	Drugs, carcinogens	Rare	No conclusive studies	–
CYP3A5	<1	Drugs	<i>CYP3A5*3</i>	No conclusive studies	–

# Summary of clinical effects relevant to genetic polymorphisms in CYPs and drug dosage

Table 3  
Impact of P450 polymorphisms on xenobiotic toxicity and drug treatment in vivo

Polymorphic enzyme	Decreased clearance in PMs	Effect on activation of environmental agents	Adverse effects in PMs	Reduced prodrug activation in PMs
CYP1B1	PAH, estrogens	Different susceptibility for PAH and estrogenic carcinogenicity		
CYP2A6	Nicotine	Affecting smoking behaviour	Higher nicotine levels	
CYP2C9	<i>S</i> -warfarin Phenytoin Tolbutamide NSAIDs	Not known	Bleedings Ataxia Hypoglycemia	Losartan
CYP2C19	Omeprazole Diazepam	Not known	Severe sedation	Proguanil
CYP2D6	Tricyclic antidepressants SSRIs Haloperidol Perphenazine Zuclopenthixol Perhexiline Antiarrhythmic drugs Phenformin	Not known	Cardiotoxicity Nausea  Neuropathy Arrhythmias Lactic acidosis	Encainide Codeine Ethylmorphine

Ingelman-Sundberg M. Genetic susceptibility to adverse effects of drugs and environmental toxicants. The role of the CYP family of enzymes. *Mutat Res.* 2001 Oct 1;482(1-2):11-9.

# Variant forms of the CYPs of highest importance for the metabolism of drugs and other xenobiotics

Table 2  
Major human polymorphic cytochrome P450 enzymes<sup>a</sup>

Enzyme	Major variant	Mutation	Consequence	Allele frequency (%)	
				Caucasians	Oriental
CYP2A6	<i>CYP2A6*2</i>	L160H	Inactive enzyme	1-3	0
	<i>CYP2A6*3</i>	2A6/2A7	Not known	0	0
	<i>CYP2A6*4</i>	Gene deletion	No enzyme	1	15
	<i>CYP2A6*5</i>	G479L	Defect enzyme	0	1
CYP2C9	<i>CYP2C9*2</i>	R144C	Reduced affinity for p450 reductase	8-13	0
	<i>CYP2C9*3</i>	I359L	Altered substrate specificity	7-9	2-3
CYP2C19	<i>CYP2C19*2</i>	Altered splicing site	Inactive enzyme	13	23-32
	<i>CYP2C19*3</i>	Stop codon	Inactive enzyme	0	6-10
CYP2D6	<i>CYP2D6*2xn</i>	Gene duplicate	Increased activity	1-5	0-2
	<i>CYP2D6*4</i>	Defective splicing	Inactive enzyme	12-21	1
	<i>CYP2D6*5</i>	Gene deletion	No enzyme	4-6	6
	<i>CYP2D6*10</i>	P34S, S486T	Unstable enzyme	1-2	50
	<i>CYP2D6*17</i>	T107I, R296C, S486T	Reduced affinity for substrates	0	In Blacks, 34% allele frequency
CYP2E1	<i>CYP2E1*2</i>	R76H	Less enzyme expressed	0	1
	<i>CYP2E1*3</i>	V389I	No effects	<1	0
	<i>CYP2E1*4</i>	V179I	No effects	<1	N.D.
CYP3A4	<i>CYP3A4*2</i>	S222P	Higher $K_m$ for substrate	3	0
	<i>CYP3A4*3</i>	M445T	Unknown	0	<1
	<i>CYP3A4*4</i>	I118V	Decreased	0	<1
	<i>CYP3A4*5</i>	P218R	Decreased	0	<1
	<i>CYP3A4*6</i>	831 insA	Decreased	0	<1

<sup>a</sup> See footnote 1 for details and literature references.

# Ethnic distribution of the most common variant alleles of CYP2D6

**Table 3** Major human polymorphic variant *CYP2D6* alleles and their global distribution. For a complete list, see <http://www.imm.ki.se/cypalleles/cyp2d6.htm>

Major variant alleles	Mutation	Consequence	Allele frequencies (%)			
			Caucasians	Asians	Black Africans	Ethiopians and Saudi Arabians
<i>CYP2D6*2xn</i>	Gene duplication/multiduplication	Increased enzyme activity	1–5	0–2	2	10–16
<i>CYP2D6*4</i>	Defective splicing	Inactive enzyme	12–21	1	2	1–4
<i>CYP2D6*5</i>	Gene deletion	No enzyme	2–7	6	4	1–3
<i>CYP2D6*10</i>	P34S, S486T	Unstable enzyme	1–2	51	6	3–9
<i>CYP2D6*17</i>	T107I, R296C, S486T	Altered affinity for substrates	0	0	20–35	3–9

Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.* 2005;5(1):6-13.

**Table 1 Major substrates and inhibitors for CYP2D6***Major classes of drugs as substrates*

Antidepressants  
Tricyclic antidepressants  
Serotonin reuptake inhibitors  
Neuroleptics  
Beta-blockers  
Antiarrhythmics

*Specific drugs as substrates*

Alprenolol  
Amiflamine  
Aprindine  
Atenolol  
Bufuralol  
Bupranolol  
Chlorpropamide  
Clomipramine  
Clozapine  
Codeine  
Debrisoquine  
Desimipramine  
Desmethylcitalopram  
Dextromethorphan  
Dihydrocodeine  
Encainide  
Ethylmorphine  
Flecainide  
Flunarizine  
Fluperlapine  
Guanoxan  
Haloperidol  
Hydrocodone  
Imipramine  
Indoramin  
Maprotiline  
Methoxyamphetamine  
Methoxyphenamine  
Metiamide  
Metoprolol  
Mexiletine  
Nortriptyline

Ondansetron  
Otycodone  
Perhexiline  
Perphenazine  
Phenacetin  
Phenformin  
Propafenone  
Propranolol  
Quinidine  
Risperidone  
Thioridazine  
Timolol  
Tomoxetine  
Tropisetron  
Zuclopenthixol

*Inhibitors, drugs*

Chinidin  
Fluoxetine  
Levomepromazine  
Lobelin  
Methadone  
Paroxetine  
Quinidine  
Trifluoperidol

*Inhibitors, alkaloids<sup>a</sup>*

Ajmalicine  
Ajmalicine  
Berberine  
Coniine  
Ergotamine  
Gramine  
Hamaline  
Laudanosine  
Sempervirine  
Vincamine  
Vinblastine

<sup>a</sup>Several of these alkaloids have not been evaluated as substrates. See<sup>56</sup> for a complete list of substrates and inhibitors.

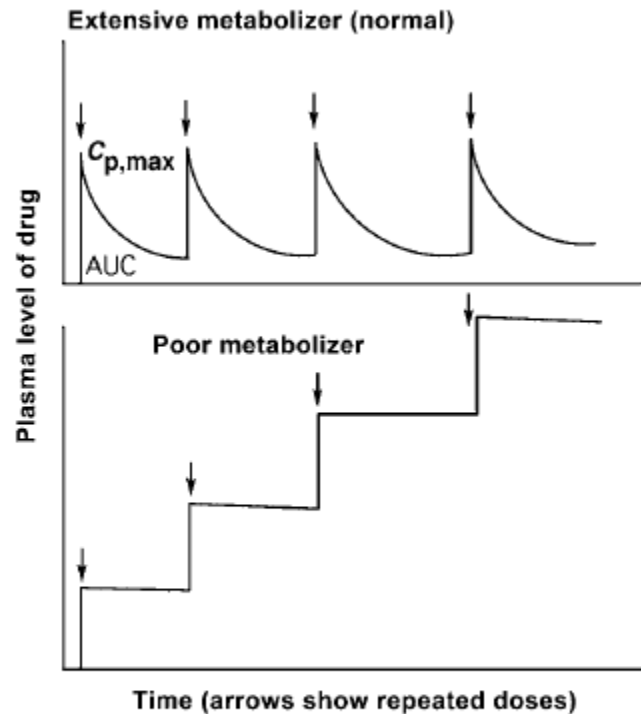
# CYP2D6 drug substrates and inhibitors

CYP2D6 has a very high affinity for alkaloids

Enzyme expression not regulated by any known environmental agent and not inducible by known hormones

Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.* 2005;5(1):6-13.

# Significance of variability in levels of a single CYP (P450)

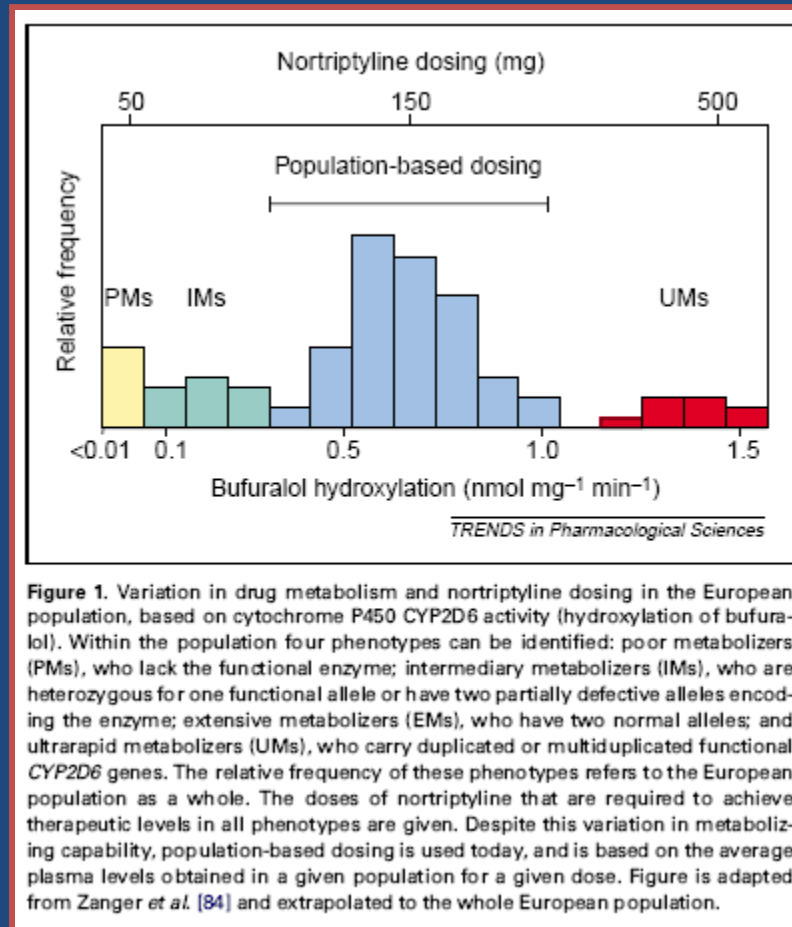


**Figure 3.** Effect of variation in human P450 activities on pharmacokinetics. P450 indicates cytochrome P450; AUC, area under the curve.<sup>2</sup>

Upper panel – level of drug in the plasma maintained in a range that yields desired pharmacological effect.

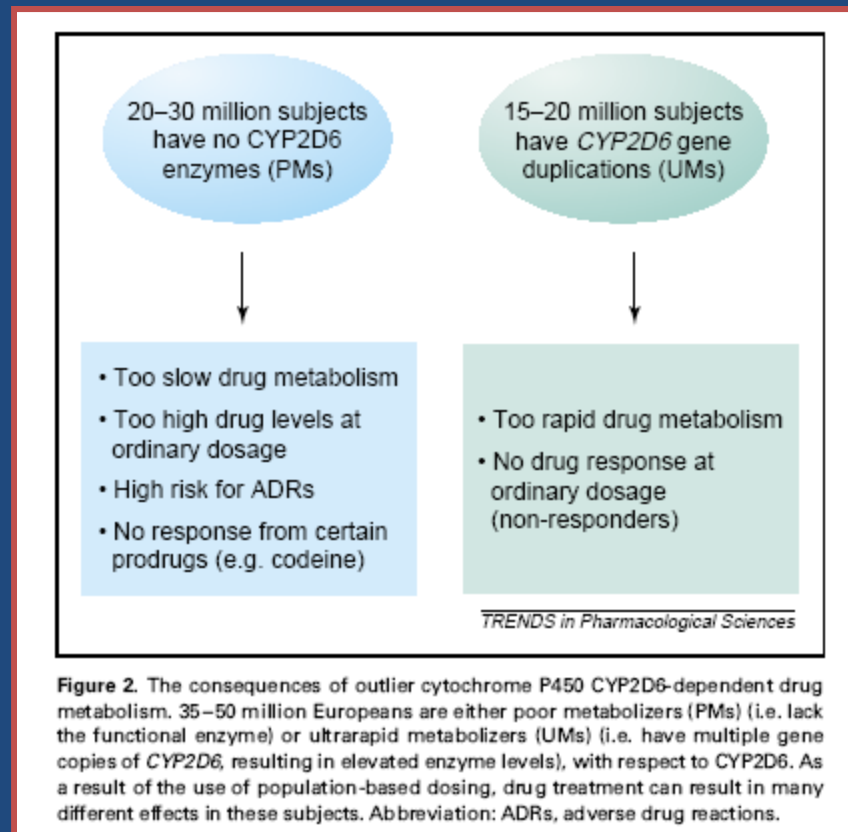
Lower panel - individual deficient in the particular P450 shows very limited metabolism of same dose of drug, leading to a progressive increase in plasma drug levels with repeated dosing.

# Variation in drug metabolism and nortriptyline dosing based on CYP2D6 activity



Ingelman-Sundberg M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends Pharmacol Sci.* 2004 Apr;25(4):193-200.

# Consequences for poor metabolizers (PMs) and ultrarapid metabolizers (UMs) of CYP2D6-dependent drugs at ordinary drug doses



Ingelman-Sundberg M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. Trends Pharmacol Sci. 2004 Apr;25(4):193-200.

# Dosage of commonly used antipsychotics is dependent on the *CYP2D6* genotype

Table 4 Approximate dose adjustments according to the *CYP2D6* phenotype as based on the meta-analysis by Kirchheiner *et al.*<sup>45</sup> Recommended dosages in relation to recommended one are presented for the poor metaboliser (PM), intermediate metaboliser (IM), efficient metaboliser (EM) and ultrarapid metaboliser (UM) phenotypes

	PM	IM	EM	UM
<i>Antidepressants</i>				
Imipramine	30	75	130	180
Doxepin	35	77	120	170
Maprotiline	35	77	120	170
Trimipramine	37	83	125	175
Desipramine	40	76	117	165
Nortriptyline	48	90	115	155
Clomipramine	60	85	112	145
Paroxetine	65	90	108	143
Venlafaxine	68	85	105	130
Amitriptyline	70	90	105	135
Mianserin	70	87	110	135
<i>Antipsychotics</i>				
Perphenazine	30	80	130	170
Thioridazine	37	82	127	165
Olanzapine	50	100	120	155
Zuclopenthixol	55	85	115	142
Aripiprazole	60	85	112	130
Flupentixol	68	80	117	135
Haloperidol	67	90	108	126

For the antidepressants mirtazapine, moclobemide, fluoxetine, maprotiline, bupropion, nefazodone, citalopram and sertraline as well as the antipsychotics perazine, risperidone, pimozide, clozapine, levomepromazine, olanzapine no significant dose adjustments based on *CYP2D6* was recommended.

Ingelman-Sundberg M. Genetic polymorphisms of cytochrome P450 2D6 (*CYP2D6*): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.* 2005;5(1):6-13.

# View of complexity of events involved in cell toxicity

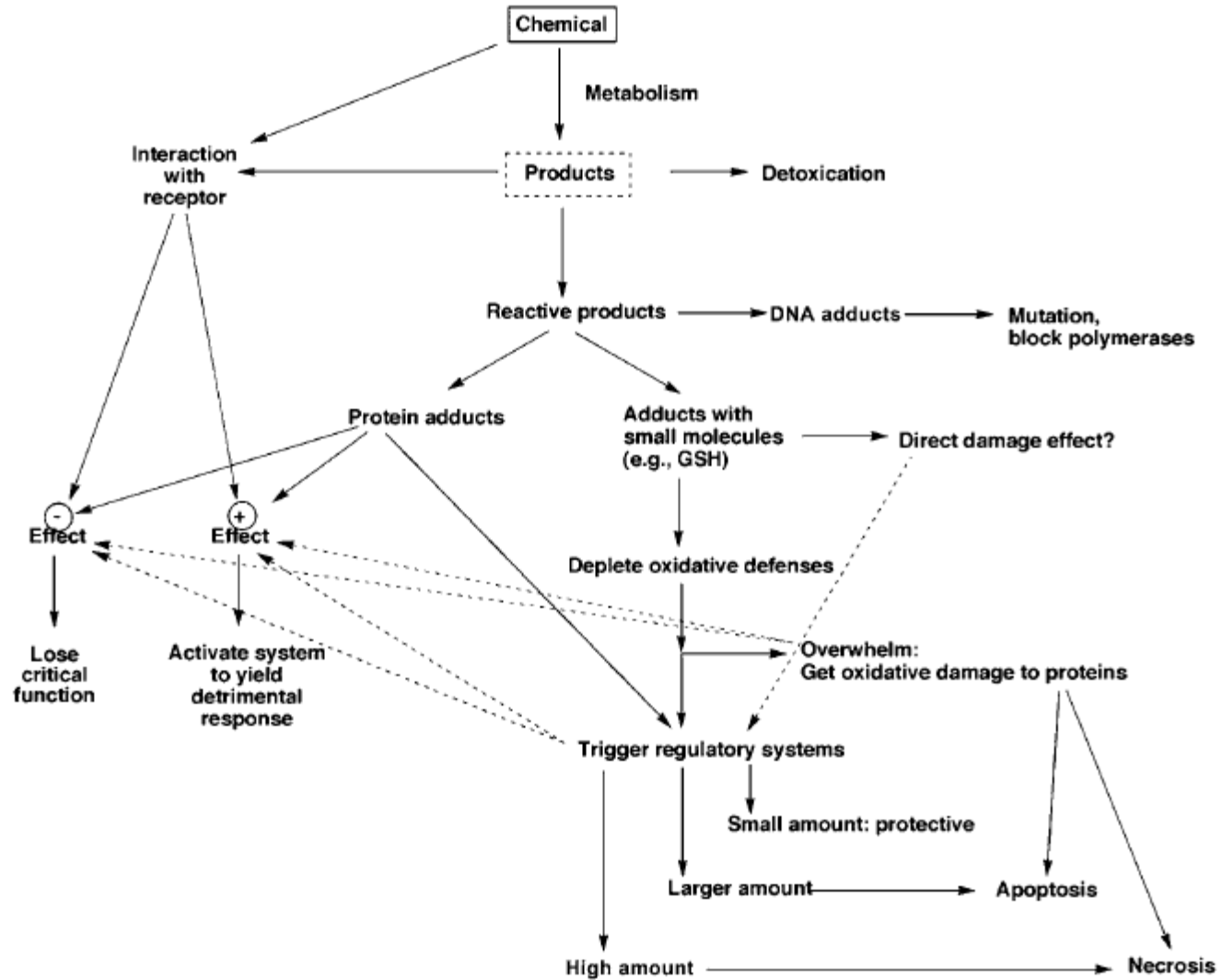
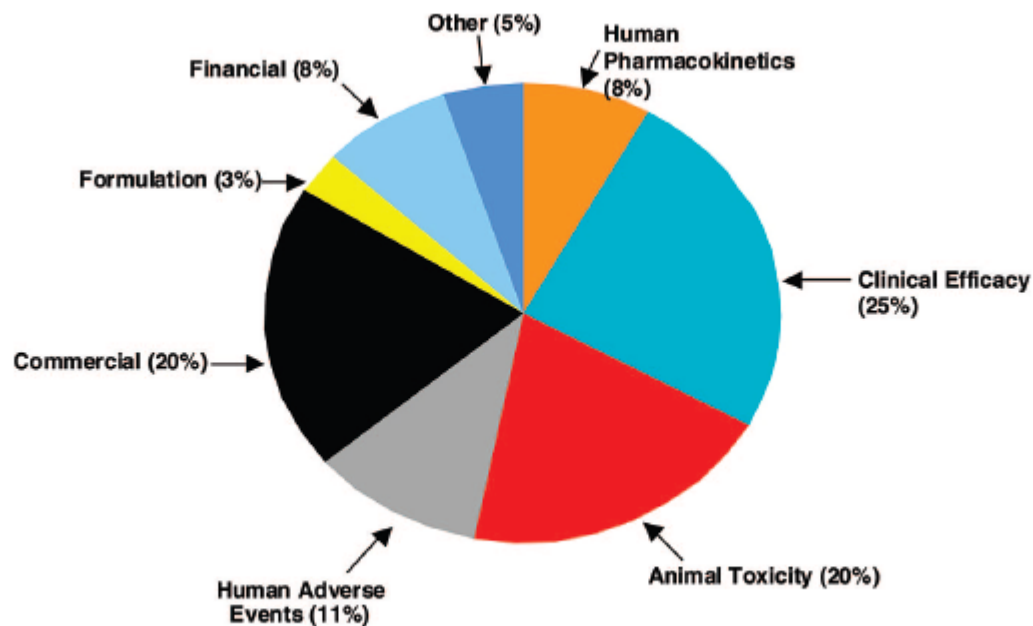


Figure 7. Biological events possibly relevant to chemical toxicity. GSH, glutathione.

In comparison to 25 years ago, fewer drugs fail in development due to pharmacokinetic problems in humans



**Figure 1.** Reasons for the termination of drug candidates during development, based upon surveys of the pharmaceutical industry (ca. 2000) (12). See also Table 13 of ref 14.

# Tools of the trade for discovery of which CYP metabolizes your drug of interest

In Vitro Assay Systems - substrates, inhibitors - for a 'specific' CYP

In Vitro Assay Systems with substrates, specific for a CYP

Purified CYPs, B5, Reductase

Reconstituted Systems with the above

Microsomes from insects, COS cells, CHO cells engineered to express a specific CYP.

COS, CHO, HepG2 cell lines which express a specific CYP

Polyclonal and Monoclonal antibodies to specific CYPs

Adenoviral systems to overexpress a CYP

Antisense adenoviral systems to decrease expression of a CYP

SiRNAs to decrease expression of a CYP

CYP knockout mice

Humanized CYP mice

NADPH P450 Reductase knockout mice

CYP on a chip - personalized medicine

*Many of the above being currently extended to polymorphic forms of a CYP*