5 Species Differences of Drug-Metabolizing Enzymes

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5.1 OVERVIEW

This chapter introduces the reader to the basic differences in drug-metabolizing enzymes (DMEs) between humans and the most commonly employed preclinical animal models. The phenotypic differences in metabolism that are observed between species are rooted in each species genotype; however, it is generally not possible to determine the observed phenotype from the specific genotype. These genotypic differences are largely driven by the different dietary and environmental pressures that each species has been exposed to and ultimately lead to an expressed specific phenotype. This chapter explores both phenotypic and genotypic differences among species in order to give an overview of the many factors that alter the metabolic response between different organisms.

The ability of an organism to respond to the numerous potentially toxic chemicals that it encounters is controlled by a “xenobiotic response,” which can roughly be broken down into two parts, xenobiotic-sensing receptors and DMEs. The xenobiotic-sensing receptors covered here are the aryl hydrocarbon receptor (AHR), the constitutive androstane receptor (CAR), and the pregnane X receptor (PXR). These receptors control the expression of the DMEs that functionally modify xenobiotics in an attempt to detoxify and excrete them from the body. The enzymes covered in this chapter are the cytochrome P450s (CYPs), the uridine 5′-diphospho-glucuronosyltransferases (UGTs), the sulfotransferases (SULTs), and the flavin-containing monooxygenases (FMOs).

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Although the overall goal of the "xenobiotic response" is the same for all mammalian species, the systems involved have diverged considerably over time. This divergence has lead to differences in the primary genetic sequence of each gene resulting in differences in basal enzyme expression and induction, altered enzyme kinetics and substrate specificity, and ultimately the observed differences in the absorption, distribution, metabolism, excretion (ADME), and toxicity of xenobiotics.[1]

5.2 INTRODUCTION

Animal models are commonly used in preclinical drug development to predict the pharmacokinetic and drug metabolism behaviors of new chemical entities (NCEs) in humans. One of the challenges drug metabolism scientists are facing is the extrapolation of metabolic data from animal studies to humans to incorporate into the overall ADME data set. Interspecies differences in drug metabolism have been recognized for many years, with some of the earlier studies investigating the ratio of ortho- to para-hydroxylation of aniline and 1,1′-biphenyl across species. These studies noted differences not only between species but also between different strains of animals, males and females, and the young and old [1–3].

The challenge in predicting human pharmacokinetics and drug metabolism from preclinical animal models is twofold in that there are significant differences in drug metabolism between species (the focus of this chapter) as well as a large amount of variability among human beings due in part to differences in enzyme and receptor expression levels and single nucleotide polymorphisms (SNPs). In order to make an informed decision as to which preclinical species will best predict human drug metabolism, it is important to gain a better understanding of the underlying mechanisms that explain species differences in drug metabolism. These differences are highly related to enzyme (isoform) composition, gene regulation, protein expression, and catalytic activities of the DMEs. The consequences of which can include altered bioavailability, changes in biological half-life, altered prodrug activation, and differential formation of active and/or toxic metabolites [4–7].

This chapter aims to provide the reader with a basic knowledge of the differences between preclinical species for the enzymes most critical in drug metabolism. The most important enzyme system for drug metabolism is the CYP superfamily, in particular, the CYP1, CYP2, and CYP3 families [8–10], and the bulk of the chapter focuses on these enzymes. The UGTs, SULTs, and FMOs are also covered but in lesser detail. Understanding species differences in drug metabolism is helpful for scientists to select the animal models from which the metabolism data can be most accurately extrapolated to humans. Unlike certain physiologic parameters, such as blood circulation and the ratio of organ weight to body weight, for which one can apply an allometric relationship across species in order to predict human pharmacokinetics from preclinical data, no such relationship can be applied to the biochemical parameters that govern the differences seen in the function of DMEs across species [11]. Therefore, it can be generally stated that the more extensively an NCE is metabolized, the more inaccurate

[1]For the purpose of this chapter, all gene names for human, monkey, and dog are represented in all-capitalized letters. Gene names for rodents have only the first letter capitalized. Protein names are represented by all-capitalized letters for all species.

the empirical approach of allometric scaling in predicting human pharmacokinetics. In a retrospective analysis, the accuracy of quantitative approaches for the prediction of human pharmacokinetics was determined using 50 compounds for which extensive preclinical data as well as clinical data were available. One of the major conclusions from this analysis was that when a compound was cleared almost exclusively by P450-mediated pathways, scaling from *in vitro* human liver microsome (HLM) data was as predictive as single-species scaling of *in vivo* clearance data from rats, dogs, or monkeys [12].

In order to give the reader an overview of the biochemical basis for the functional differences between the major DMEs that are observed across species, the authors have attempted to focus on four major factors that differ between species for each enzyme subfamily discussed: species differences in inducible enzyme expression, similarity and dissimilarity of primary protein sequence, quantitative and qualitative differences in enzyme expression, and substrate specificity for the metabolism of clinically used drugs and other xenobiotics. The primary protein sequence analysis comes in the form of an accompanying table comparing the percentage amino acid identity for each subfamily discussed in the text (Tables 5.1–5.12). All the amino acid sequences used for the tables were collected from the UniProt database [13] (http://www.uniprot.org/), and the percentage homology scores were generated using the ClustalW2 [14] sequence analysis tool from the European Bioinformatics Institute website (http://www.ebi.ac.uk/Tools/clustalw2/). Sequences were collected and aligned for human, monkey, dog, rat, mouse, rabbit, guinea pig, and hamster. The tables are limited to sequences that were deposited in the UniProt database and therefore are not representative of every known DME or of every DME discussed in the text. Instead of being all encompassing, these tables are meant as a reference to give the reader an idea of the homology between the different enzyme families and subfamilies at the amino acid level while maintaining consistency with a single database in order to avoid some of the idiosyncrasies that arise during the automated/manual curation of sequence databases by different sources. It should also be noted that the amino acid identity does not necessarily translate into catalytic specificity as a single amino acid substitution is sufficient to alter substrate specificity [15], and many considerations must be made when attempting to determine the appropriate preclinical species for drug metabolism studies. These considerations include differences in the expression levels and inducibility of individual DMEs, tissue- and gender-specific expression, and differences in substrate specificity, as well as factors that may not be related to drug metabolism at all. Turpeinen *et al*. note that if hepatic metabolism were the only factor in choosing a preclinical species, in many cases, the rat is unlikely to be an appropriate model even though it is a very common nonprimate preclinical species; however, it might also be argued that there is no global need for selecting the animal model that is metabolically closest to man. For example, in studies designed for establishing safety margins, one could select an animal model that is likely to result in high amounts of metabolites and potentially improve the safety factors for risk analyses [6].

5.3 SPECIES DIFFERENCES IN ENZYME INDUCTION

Many of the DMEs display inducible expression levels in what is considered an adaptive metabolic response. This inducible expression is governed principally by three

TABLE 5.1 Percentage Amino Acid Homology for CYP1A Subfamily in Various Species

	Human CYP1A1	Human CYP1A2	Cynomolgus Monkey CYP1A1	Cynomolgus Monkey CYP1A2	Rhesus Monkey CYP1A1	Dog CYP1A1	Dog CYP1A2	Rat Cyp1a1	Rat Cyp1a2	Mouse Cyp1a1	Mouse Cyp1a2	Rabbit Cyp1a1	Rabbit Cyp1a2	Guinea Pig Cyp1a1	Guinea Pig Cyp1a2	Hamster Cyp1a1	Hamster Cyp1a2
Human CYP1A1	100	71	94	71	94	81	67	79	66	80	66	76	67	77	66	79	65
Human CYP1A2	71	100	71	92	71	72	79	66	73	67	71	69	77	67	76	66	72
Cyno CYP1A1	94	71	100	72	99	82	67	78	66	79	66	76	68	77	66	77	65
Cyno CYP1A2	71	92	72	100	72	72	80	68	75	68	73	70	79	68	77	66	74
Rhesus CYP1A1	94	71	99	72	100	82	67	78	66	79	66	76	68	77	66	77	65
Dog CYP1A1	81	72	82	72	82	100	73	76	65	77	65	77	68	75	67	76	66
Dog CYP1A2	67	79	67	80	67	73	100	63	71	63	70	64	75	64	71	62	71
Rat Cyp1a1	79	66	78	68	78	76	63	100	68	93	67	76	65	76	64	86	65
Rat Cyp1a2	66	73	66	75	66	65	71	68	100	69	93	63	73	65	73	66	88
Mouse Cyp1a1	80	67	79	68	79	77	63	93	69	100	70	76	66	76	64	87	68
Mouse Cyp1a2	66	71	66	73	66	65	70	67	93	70	100	63	73	64	74	65	88
Rabbit Cyp1a1	76	69	76	70	76	77	64	76	63	76	63	100	71	72	65	75	62
Rabbit Cyp1a2	67	77	68	79	68	68	75	65	73	66	73	71	100	65	73	64	71
Guinea Pig Cyp1a1	77	67	77	68	77	75	64	76	65	76	64	72	65	100	69	75	63
Guinea Pig Cyp1a2	66	76	66	77	66	67	71	64	73	64	74	65	73	69	100	63	73
Hamster Cyp1a1	79	66	77	66	77	76	62	86	66	87	65	75	64	75	63	100	68
Hamster Cyp1a2	65	72	65	74	65	66	71	65	88	68	88	62	71	63	73	68	100

transcription factors: AHR, a ligand-activated member of the Per-Arnt-Sim superfamily [16], and CAR [17] and PXR [18,19], two members of the nuclear receptor superfamily. These three receptors work in concert to alter the expression of a separate but overlapping set of DME genes in response to various xenobiotics. The DME genes controlled by these receptors appear to be relatively consistent across species. The AHR has been historically known as a regulator of xenobiotic metabolism and a mediator of dioxin toxicity but it has also been implicated in many other physiologic roles [20,21]. The AHR is generally associated with the induction of the CYP1 genes, but it is also believed to regulate the expression of some of the UGT1A enzymes [22,23]. Regulation of these genes by the AHR occurs by heterodimerization of the AHR with the aryl hydrocarbon receptor nuclear translocator (ARNT) [24] and by binding to dioxin response elements (DREs) upstream of target genes [25]. Although CAR and PXR seem to be more intimately linked to the overall xenobiotic response than AHR, they have also been implicated in physiologic processes, most notably, the regulation of hepatic energy metabolism [26]. CAR and PXR regulate the expression of many CYP2B, CYP2C, CYP3A, UGT, and SULT genes by forming a heterodimer with the retinoid X receptor (RXR) and binding to a number of different promoter elements upstream of their target genes [27].

Although the battery of DME genes regulated by these receptors is fairly well conserved across species, there are considerable species differences in the ligands that activate the receptors. The ingestion of polycyclic aromatic hydrocarbons (PAHs) and β-naphthoflavone in rat, mouse, monkey, and dog leads to an increase in the level of CYP1A proteins in numerous tissues, such as liver, lung, and intestine [28–30]. The human CYP1B1 gene is also induced by dioxin [31]. Omeprazole has been reported to induce CYP1A2 in man by activating AHR, but the mechanism is not fully understood. The induction of Cyp1a genes by omeprazole does not occur in rat and mouse and is one of the few examples of species-specific activation of AHR [32]. The AHR has also shown considerable species differences in its affinity for dioxin. Guinea pigs show a 1000-fold lower sensitivity to dioxin-induced toxicity when compared to hamster [33]. There are two Ahr alleles in mouse Ahr^b and Ahr^d. The Ahr^d encodes a receptor with 10-fold lower affinity for dioxin [20,34]. The human AHR shows a further 10-fold reduction in affinity for dioxin when compared to the mouse Ahr^d allele [35]. Therefore, the C57BL/6 strain of mice that express the AHR^b enzyme [36] is likely not to be a good model for investigations into human AHR activation.

The nuclear receptors CAR and PXR show a greater degree of alternative ligand binding across species compared to AHR. Phenobarbital is a potent activator of CAR in most species but it does not physically bind to CAR, instead it activates CAR by inducing the translocation of the receptor from the cytoplasm to the nucleus [37]. Lack of binding to CAR is probably the reason why activation by phenobarbital is so well conserved among species. CAR ligands, on the other hand, show a much wider array of effects on the receptor. For example, 1,4-bis[2-(3,5-dichloropyridyloxy)]benzene (TCPOBOP) is a potent activator of mouse CAR but not human or rat [38], 2,4,6-triphenyldioxane-1,3 (TPD) is specific to rat [39], and 6-(4-chlorophenyl)imidazo[2,1-*b*][1,3]thiazole-5-carbaldehyde *O*-(3,4-dichlorobenzyl)oxime (CITCO) is specific to human [40]. The antifungal clotrimazole is an inverse agonist of human CAR but an agonist of rat CAR [41]. The antinausea agent meclizine is also an inverse agonist of human CAR but is a potent agonist of mouse CAR [42]. PXR can bind to a large variety of chemicals because of its large and flexible ligand-binding pocket [43].

Rifampicin is a potent PXR ligand in humans, dogs, and rabbits [30,44,45]. In mice and rats, the prototypical PXR agonists are dexamethasone and pregnenolone 16α-carbonitrile (PCN) [44,45]. Troglitazone is an activator of human PXR and inducer of human CYP3A4 but does not induce Cyp3a in rodent models or activate rodent PXR [46]. Clotrimazole is an agonist of human, mouse, and rat PXR [41,47], and hyperforin, an active component of St. John's Wort, is an agonist of human PXR but not rat [48].

As discussed in greater detail later, the evolution of adaptive responses to xenobiotics is likely to be greatly influenced by diet. Phylogenetic analyses of CAR and PXR support this hypothesis. CAR and PXR diverged from the chicken X receptor (Cxr) and showed cross talk in the genes that they regulate and the ligands that activate them, creating some redundancy in the expression pathways that regulate DMEs in higher organisms [49] presumably as a means of responding to a larger cadre of exogenous chemicals that these organisms were exposed to. Both of these nuclear receptors show high ratios of nonsynonymous (Ka) to synonymous (Ks) substitutions (the ω-ratio) within their ligand-binding domains when compared to other members of the nuclear receptor superfamily [50]. This ratio is highly suggestive of positive selection for binding to unique ligands and is not surprising when one considers that these two receptors are the most intimately related to the expression of the DMEs most important to xenobiotic metabolism. The high ω-ratio generally equates to rapid evolution and can again likely be traced back to the evolutionary pressure that diet places on this system. In light of this, it is somewhat surprising that there is almost no variation seen in the ligand-binding domains of CAR or PXR among humans [51,52], with most of the SNPs being found within the Japanese population [53,54]. This observation suggests that there are ligands applying negative pressure to the evolution of the CAR and PXR ligand-binding domains in the human population [50]. Phylogenetic analysis of the AHR suggests that ligand binding is a function of the receptor, which arose during vertebrate evolution [20] and has likely developed as a secondary role to the many physiologic processes that the receptor has been implicated in, such as fetal vascular development [55] and cardiac hypertrophy [56].

Further functional differences in these receptors across species can be found in the differential splicing of the primary transcripts. CAR has been shown to generate splice variants in mouse [57] and human [58–62]. The alternatively spliced mouse receptor lacked the ability to bind DNA and did not show any transcriptional or inhibitory properties [57]. A larger number of alternatively spliced CAR transcripts have been described in humans, a few of which have shown functional differences when compared to the reference form of the receptor, including a switch from constitutive activity to ligand-activated activity for CAR2 and CAR3 as well as the ability of CAR2 to recognize unique ligands [63–65]. A number of human PXR splice variants have also been described [66]. The most abundant variant, hPXR.2, is missing 37 amino acids from the ligand-binding pocket and displays significantly diminished ligand-activated transcriptional activity [67,68]. The intronic sequences that signal for the alternative exon splicing do not appear to be well conserved across species, for example, the splice acceptor site for CAR2 has been shown to be conserved across human, rhesus monkey, and guinea pig, but not in mouse, rat, or marmoset [64]. These observations suggest that changes in the genetic code that control pre-mRNA processing are another means by which the diversity of xenobiotic response systems and the ability to adapt to environmental pressures can be increased in a species.

5.4 CYTOCHROME P450s

5.4.1 Overview

The CYPs are hemoproteins that are among the most ancient proteins known and are expressed in almost all living organisms today, from archaebacteria to humans [69]. They are a fundamental enzyme family for the anabolic and catabolic metabolism of many of the endogenous chemicals required for life and also play critical roles in the biotransformation (bioactivation and detoxification) of a wide variety of xenobiotic chemicals [70,71]. The CYPs are believed to have evolved from a single ancestral gene 3.5 to 4.0 billion years ago [72]. Around 2.8 billion years ago, when oxygen levels began to increase dramatically in the atmosphere, the CYPs likely became involved in the detoxification of oxidative stress [1]. When plants and animals diverged ~1.2 billion years ago, it is believed that an "animal–plant warfare" began, whereby plants evolved toxic or unpalatable compounds to deter animals from eating them and in turn animals evolved new CYP genes to detoxify these chemicals [73]. The "animal–plant warfare" hypothesis is further supported by the observation that there was a large increase in the number of CYP genes following the evolutionary divergence of organisms with a gastrointestinal tract, which led to an increased exposure to xenobiotics from diet [70]. The unique selective pressure that diet places on the animal CYP superfamily and the overall xenobiotic response machinery is likely to be a major evolutionary drive of the functional differences observed between species. This is probably of particular importance for the CYP families 1, 2, and 3, which appear to be most important for xenobiotic metabolism and overall ADME [74], as well as the xenobiotic-sensing receptors that govern their expression.

The CYPs are encoded by a superfamily of genes and exhibit a high degree of structural and functional similarity across a vast array of species. They are localized in the smooth endoplasmic reticulum of the cell and distributed throughout the body, particularly, in the liver, intestine, nasal epithelia, and skin [9,75]. The liver and intestine are the predominate sites for P450-mediated drug elimination and are also a major site of drug–drug interactions (DDIs). The CYPs have been characterized in many species of organisms, including bacteria, fungi, plants, fish, and mammalian systems [76]. There are more than 6500 known CYP enzymes organized into 708 families, of these approximately 2279 among 99 families exist in animals. The approximately 50 CYP enzymes described in man have the most clinical relevance, in particular, approximately the 22 genes in families 1, 2, and 3 that are responsible for the majority of the metabolism of drugs and environmental pollutants [74]. The CYP enzymes from other families are largely involved in endogenous processes, particularly, biosynthesis of hormones, fatty acids, and vitamins [77]. Notably, microbial CYPs are also of clinical importance due mainly to the metabolic capacity that exists in the microflora of the gut. Differences in the composition of these microbes can account for some of the intra- and interindividual differences in drug metabolism and can lead to unexpected metabolic outcomes [78,79]. Gut microflora have been implicated in the metabolism of a number of different drugs including sulfasalazine, sulfinpyrazone, metronidazole, digoxin, isosorbide, flucytosine, and levodopa [1,80].

There are a number of animal species that have been used as preclinical models of drug metabolism; monkey, dog, rat, and mouse see the most widespread use, while rabbit, guinea pig, and hamster are used to a significantly lesser degree. There

are a number of observed differences in the CYPs across these species, including nucleotide/protein sequence, regulation, genetic polymorphism, substrate catalytic specificity, and inhibitor selectivity. 7-Ethoxycoumarin *O*-deethylase (ECOD) activity is catalyzed by several CYP isoforms and can be used as a general measure of xenobiotic-metabolizing capacity [81,82]. This method was applied in an *in vitro* study comparing overall CYP activity between human and several preclinical species, using liver microsomes. The result of the study showed that when compared to humans, ECOD activity was approximately 8.6%, 66.7%, 622%, 1086%, and 508% for mouse, rat, rabbit, dog, and monkey, respectively [6]. This result demonstrates the large range in activity levels that can be found between species and serves as a good example as to why it is valuable to have a good mechanistic understanding of the individual enzymes across species and how they differ in substrate specificity and metabolite formation.

5.4.2 CYP1A

The CYP1A subfamily (Table 5.1) consists of two members, CYP1A1 and CYP1A2. These enzymes have been extensively studied because of their importance in the bioactivation of mutagens and carcinogens. CYP1A activity can be monitored using an ethoxyresorufin *O*-deethylase (EROD) assay [83]. EROD activity in mouse, rat, rabbit, dog, and monkey liver microsomes has been shown to be approximately 374%, 102%, 413%, 630%, and 983%, respectively, of the activity found in humans [6]. The CYP1A enzymes are responsible for the species-specific metabolism of caffeine, where in humans the major metabolite is paraxanthine generated through N3-demethylation. Monkeys generate mostly theophylline by N7-demethylaltion, while rat and mouse favor C8-hydroxylation to 1,3,7-trimethyluric acid, and rabbits show approximately equal formation of paraxanthine, theophylline, 1,3,7-trimethyluric acid, and theobromine [84].

CYP1A1 is responsible for bioactivation of many environmental carcinogens, such as the PAHs benzo[*a*]pyrene and 3-methylchlanthrene, to highly carcinogenic diol epoxides [85]. The catalytic activities of the CYP1A enzymes are well conserved across mammals. In humans, CYP1A1 is not constitutively expressed (or its basal expression is very low) but is instead expressed only after induction, likely through the AHR pathway [86]. Since CYP1A1 is expressed in very low levels in human liver, the specific activity for the metabolism of PAHs in rodents is more efficient than the activity seen in uninduced human livers [87–89].

CYP1A2 is expressed almost exclusively in the liver of humans, rats, and mice [90,91]. In contrast, monkeys and dogs express CYP1A2 at low levels in the liver in the absence of treatment with an inducer [30,92]. The functional characteristics of CYP1A2 are well conserved across species [4]. In contrast to PAHs, carcinogenic heterocyclic amines are metabolized predominately by CYP1A2 [93,94]. For example, carcinogenic 2-amino-3,8-dimethylimidazol[4,5-*f*]quinoxaline (MeIQx) is metabolized in a species-specific pattern. Rodent CYP1A2 catalyzes both C- and N-oxidation of MeIQx, whereas human CYP1A2 only metabolizes N-hydroxylation at the amine group (a bioactivation pathway to carcinogenicity) [95]. In contrast, the cynomolgus monkey is resistant to MeIQx-related carcinogenicity due to low basal expression levels of CYP1A2 [96]. Therefore, the monkey is not a valuable model for comparison to human.

Human CYP1A2 accounts for 13% of the total liver CYP content and is involved in the metabolism of 4% of drugs in the market, including phenacetin, tacrine, ropinirole, acetaminophen (APAP), riluzole, theophylline, and caffeine. Furafylline is a selective inhibitor of CYP1A2 in humans, mice, rats, and dogs but is not an inhibitor of CYP1A2 in monkeys. [9,97]. Phenacetin and caffeine have been commonly used as the marker substrates for CYP1A2 in several species. Although there is a strong similarity in amino acid sequence for CYP1A2 and similar catalytic activities toward many drugs have been demonstrated between humans and many animal species, the expression of these enzymes varies considerably among species. For example, dogs and monkeys have low expression levels of the enzyme in the liver and may not serve as good models of CYP1A2-mediated drug metabolism and toxicity in humans in the drug development process [98].

5.4.3 CYP1B

CYP1B1 is the only member of the CYP1B family (Table 5.2). Human CYP1B1 is constitutively expressed in normal tissues, such as lung, liver, heart, brain, kidney, placenta, and prostate [31]. Expression of CYP1B1 is species dependent. Rat Cyp1b1 is expressed in liver and lung, while mouse Cyp1b1 is present in testis, kidney, lung, brain, and heart but not in liver [90,99]. CYP1B1 is differentially expressed within the tumor microenvironment of many cancers, showing much higher expression in tumor cells compared to the surrounding tissues [100,101]. It is also an important factor in determining risk associated with hormone-mediated cancers and catalyzes estrogens to form active 4-hydroxylated derivatives that may cause breast cancer [102]. Similar to CYP1A1, human CYP1B1 and rodent CYP1B1 can bioactivate carcinogenic PAHs to DNA-reactive species associated with toxicity, mutagenesis, and carcinogenesis [103]. Currently, little is known about the activity of CYP1B1 in most preclinical species, and further investigations are necessary.

5.4.4 CYP2A

Table 5.3 shows the percentage amino acid homology for the CYP2A subfamily across species. The human CYP2A subfamily includes CYP2A6, CYP2A7, and CYP2A13. Human CYP2A6 is expressed predominately in liver where it accounts for about 4% of total hepatocyte CYP content [9] but has also been found in the nasal mucosa, trachea, lung, and skin [104,105], while CYP2A13 is mainly expressed in the respiratory

TABLE 5.2 Percentage Amino Acid Homology for CYP1B Subfamily in Various Species

	Human CYP1B1	Cynomolgus Monkey CYP1B1	Dog CYP1B1	Rat Cyp1b1	Mouse Cyp1b1
Human CYP1B1	100	94	84	80	81
Cyno CYP1B1	94	100	86	84	83
Dog CYP1B1	84	86	100	80	81
Rat Cyp1b1	80	84	80	100	93
Mouse Cyp1b1	81	83	81	93	100

TABLE 5.3 Percentage Amino Acid Homology for CYP2A Subfamily in Various Species

	Human CYP-2A6	Human CYP-2A7	Human CYP-2A13	Cyno-molgus Monkey CYP2A23	Cyno-molgus Monkey CYP2A24	Rhesus Monkey CYP2A23	Rhesus Monkey CYP2A24	Dog CYP-2A13	Dog CYP-2A25	Rat Cyp-2a1	Rat Cyp-2a2	Rat Cyp-2a3	Mouse Cyp2a4	Mouse Cyp2a5	Mouse Cyp2a12	Mouse Cyp2a22	Rabbit Cyp2a10	Rabbit Cyp2a11	Hamster Cyp2a8	Hamster Cyp2a9
Human CYP2A6	100	93	93	92	94	92	94	89	85	69	64	85	83	85	69	70	84	84	73	69
Human CYP2A7	93	100	91	89	93	89	93	87	83	69	65	84	82	83	70	70	82	83	72	69
Human CYP2A13	93	91	100	94	93	93	94	92	87	72	67	88	86	88	72	73	87	87	75	71
Cyno CYP2A23	92	89	94	100	93	98	93	91	87	72	68	88	85	87	72	73	86	86	75	71
Cyno CYP2A24	94	93	93	93	100	93	98	89	86	70	66	86	84	85	71	71	85	84	75	70
Rhesus CYP2A23	92	89	93	98	93	100	93	90	87	71	68	87	85	87	72	73	86	86	75	71
Rhesus CYP2A24	94	93	94	93	98	93	100	90	87	70	66	87	85	86	71	72	85	85	76	70
Dog CYP2A13	89	87	92	91	89	90	90	100	93	71	66	90	87	89	71	71	87	88	73	71
Dog CYP2A25	85	83	87	87	86	87	87	93	100	70	66	86	83	85	70	71	84	84	72	69
Rat Cyp2a1	69	69	72	72	70	71	70	71	70	100	88	71	69	70	88	86	69	69	65	83
Rat Cyp2a2	64	65	67	68	66	68	66	66	66	88	100	67	65	66	82	80	65	64	61	76
Rat Cyp2a3	85	84	88	88	86	87	87	90	86	71	67	100	94	96	72	72	86	86	76	71
Mouse Cyp2a4	83	82	86	85	84	85	85	87	83	69	65	94	100	97	69	70	84	85	74	70
Mouse Cyp2a5	85	83	88	87	85	87	86	89	85	70	66	96	97	100	70	71	86	86	76	70
Mouse Cyp2a12	69	70	72	72	71	72	71	71	70	88	82	72	69	70	100	93	69	70	65	81
Mouse Cyp2a22	70	70	73	73	71	73	72	71	71	86	80	72	70	71	93	100	69	70	65	80
Rabbit Cyp2a10	84	82	87	86	85	86	85	87	84	69	65	86	84	86	69	69	100	98	74	68
Rabbit Cyp2a11	84	83	87	86	84	86	85	88	84	69	64	86	85	86	70	70	98	100	74	68
Hamster Cyp2a8	73	72	75	75	75	75	76	73	72	65	61	76	74	76	65	65	74	74	100	63
Hamster Cyp2a9	69	69	71	71	70	71	70	71	69	83	76	71	70	70	81	80	68	68	63	100

tract [106]. Rat Cyp2a1 (female dominant) and Cyp2a2 (male dominant) are expressed in the liver [107,108], while rat Cyp2a3 is constitutively expressed in the esophagus, lung, and nasal epithelium but is absent in liver [109]. Mouse Cyp2a5 is expressed in the olfactory mucosa, liver, lung, kidney, brain, and small intestine [110]. Cyp2a4 is a predominately female isoform found in mouse liver [111], and mouse Cyp2a12 has been found in the liver but not in the olfactory mucosa [112].

The human CYP2A enzymes show different substrate specificity when compared to the CYP2A of animal species. Human CYP2A6 is involved in the metabolism of a wide range of xenobiotics, including coumarin, nicotine, cyclophosphamide, fadrozole, and aflatoxin B1 [4,113]. Activation of aflatoxin B1 to its carcinogenic/toxic AFB1 (aflatoxin B1)-8,9-epoxide and AFM1 (aflatoxin M1)-8,9-epoxide by CYP2A13 is likely to play a role in the development of lung cancer after aflatoxin inhalation [114]. CYP2A13 is also involved in the metabolism of nicotine and cotinine [115]. Unlike their human orthologs, rodent CYP2A enzymes catalyze the 7α- and 15α-hydroxylation of steroids. For example, CYP2A1 is highly specific for the catalysis of 7α-hydroxylation of testosterone, while CYP2A2 catalyzes both the 15α- and the 7α-hydroxylation of testosterone [113,116]. All CYP2A isoforms appear to catalyze coumarin 7-hydroxylation (a marker substrate) but exhibit regioselectivity of the hydroxylation at all six possible positions [117]. Using coumarin 7-hydroxylase activity as a measure of overall CYP2A function [118] in mouse, rabbit, dog, and monkey liver microsomes, it was found that these species had approximately 18%, 8.7%, 2.9%, and 75%, respectively, of the activity found in HLM preparations. In the same study, coumarin 7-hydroxylase activity in rats was found to be below the limit of quantification [6], and in a similar study, it was found to be detectable only when large amounts of substrate were used [119]. This is because in rats, coumarin metabolism occurs mainly through a 3,4-epoxidation reaction catalyzed by CYP2A1 [120]. This regioselective difference in coumarin metabolism between humans and rats also explains the differences in coumarin toxicity between the species; coumarin shows hepatotoxicity and tumor induction in rats because of the epoxide metabolite [117]. In mice, coumarin 7-hydroxylase activity is carried out by CYP2A5 [121] and varies across different strains. The DBA/1 J and DBA/2 J strains show high 7-hydroxylase activity unlike the C57BL/6 J strain that demonstrates low activity at this site [122].

5.4.5 CYP2B

The homology of the CYP2B subfamily across species is presented in Table 5.4. The human CYP2B family includes CYP2B6 and the psuedogene CYP2B7. CYP2B6 is mainly expressed in liver and is involved in the metabolism of a large number of drugs in the market, such as the anticancer drugs cyclophosphamide and tamoxifen, the anesthetics ketamine and propofol, and the procarcinogen aflatoxin B1 [117]. Using pentoxyresorufin *O*-depentylase (PROD) activity as a measurement of overall CYP2B activity [83], it was found that activity in mouse, rat, rabbit, dog, and monkey microsomes was approximately 1000%, 517%, 4827%, 3793%, and 827% of human microsomal activity, respectively [6]. In the same study, using the more specific bupropion hydroxylase assay [123,124], the CYP2B activity was found to be 47%, below quantitation limit, 1084%, 146%, and 605% for the same species compared to humans. The difference in these two assays highlights the large variability that can be seen in the metabolism of different substrates by similar enzymes. The basal expression of

TABLE 5.4 Percentage Amino Acid Homology for CYP2B Subfamily in Various Species

	Human CYP2B6	Cyno-molgus Monkey CYP2B6	Rhesus Monkey CYP2B30	Dog CYP2B11	Rat Cyp2b1	Rat Cyp2b2	Rat Cyp2b3	Rat Cyp2b12	Rat Cyp2b15	Mouse Cyp2b9	Mouse Cyp2b10	Mouse Cyp2b13	Mouse Cyp2b19	Rabbit Cyp2b4	Rabbit Cyp2b5
Human CYP2B6	100	91	91	78	75	74	67	72	72	71	75	71	74	77	75
Cyno CYP2B6	91	100	99	80	75	74	66	72	71	71	73	71	74	78	77
Rhesus CYP2B30	91	99	100	80	75	75	67	72	72	71	74	71	74	78	77
Dog CYP2B11	78	80	80	100	74	74	67	71	70	71	74	72	73	79	78
Rat Cyp2b1	75	75	75	74	100	97	76	80	80	80	90	82	82	77	76
Rat Cyp2b2	74	74	75	74	97	100	76	79	80	80	88	80	82	77	76
Rat Cyp2b3	67	66	67	67	76	76	100	71	71	76	75	76	74	68	68
Rat Cyp2b12	72	72	72	71	80	79	71	100	89	77	78	77	87	72	71
Rat Cyp2b15	72	71	72	70	80	80	71	89	100	76	78	76	86	74	72
Mouse Cyp2b9	71	71	71	71	80	80	76	77	76	100	83	89	79	72	71
Mouse Cyp2b10	75	73	74	74	90	88	75	78	78	83	100	83	81	77	75
Mouse Cyp2b13	71	71	71	72	82	80	76	77	76	89	83	100	79	72	72
Mouse Cyp2b19	74	74	74	73	82	82	74	87	86	79	81	79	100	76	74
Rabbit Cyp2b4	77	78	78	79	77	77	68	72	74	72	77	72	76	100	97
Rabbit Cyp2b5	75	77	77	78	76	76	68	71	72	71	75	72	74	97	100

CYP2B6 has been reported to be extremely variable in humans [125–127], and the gene is also highly inducible through the CAR/PXR pathway, making the extrapolation from preclinical species even more difficult.

Mouse Cyp2b9 (female) and Cyp2b10 (male) are sexually dimorphic and lack catalytic activity. The rat Cyp2b subfamily mainly includes Cyp2b1 and Cyp2b2, which have very similar substrate specificities. Both are expressed constitutively in liver and in the extrahepatic tissues, intestine, and lung. Dog CYP2B11 catalyzes the N-demethylation of dextromethorphan, N1-demethylation of diazepam, and the 4′-hydroxylation of mephenytoin [128,129]. Rat, rabbit, and dog CYP2B isoforms all metabolize steroids (androstenedione, testosterone, and progesterone); however, each of these enzymes show unique steroid hydroxylation profiles [130]. 7-Ethoxy-4-trifluoromethylcoumarin and 7-pentoxyresorufin are also metabolized by CYP2B isoforms. K_m and V_{max} values for the enzymes are conserved in liver microsomes across human, mouse, rat, dog, and rabbit species but not in monkey species [7]. Cyclophosphamide, an anticancer prodrug, is activated in the liver by 4-hydroxylation and deactivated by N-decholoroethylation, both of which were observed in rat (CYP2B1) and man (CYP2B6) [131].

5.4.6 CYP2C

The CYP2C subfamily is the largest subfamily and contains multiple isoforms in all species (Table 5.5). In humans, CYP2C isoforms make up ~20% of the total CYP content in liver [132] and are involved in the metabolism of about 16% of drugs in the market [133]. CYP2C8 and CYP2C9 are the major expressed CYP2C forms in humans (>85%), while CYP2C19 expression is low (1%) [134]. Relative to human microsomes, CYP2C activity in mouse, rat, rabbit, dog, and monkey has been found to be 0.6%, 39%, 34%, below the limit of quantitation, and 47%, respectively, by the tolbutamide hydroxylase assay (OH-TOL) [135,136] and 43%, 28%, 50%, below the limit of quantitation, and 172%, respectively, by the omeprazole 5-hydroxylase (5-OH-OME) assay [6,137].

CYP2C8 is expressed mainly in the liver but has also been detected in the kidney, adrenals, and small intestine [138]. It catalyzes the metabolism of retinol and retinoic acid, arachidonic acid, and the anticancer drug paclitaxel (a selective marker) [9,139]. CYP2C9 shows a pattern of expression that is very similar to that of CYP2C8 [138] and metabolizes many clinically important drugs including the diabetic drugs tolbutamide (a selective marker); the anticonvulsant phenytoin; the anticoagulant warfarin; many anti-inflammatory drugs, such as ibuprofen, diclofenac, piroxicam, and tenoxicam; the antihypertensive losartan; the antidiabetic glipizide; and the diuretic torasemide [140]. CYP2C19 is detected in the liver and small intestine [138], is highly polymorphic [141], and has been shown to metabolize *S*-mephenytoin (a selective marker), omeprazole, imipramine, diazepam, barbiturates, and proguanil [9,142–144].

Mouse has more than 10 Cyp2c members. These Cyp2c isoforms have similar catalytic activity in the oxidation of arachidonic acid and hydroxyeicosatetraenoic acids [145]. Among the mouse Cyp2c isoforms, CYP2C37 is the most abundant form in the liver and CYP2C29 is the most abundant in the lung, while CYP2C40 is the major CYP2C found in both kidney and intestine [145–147]. All these enzymes catalyze tolbutamide. The rat Cyp2c family includes at least seven isoforms. They are all expressed in the liver and oxidize dihydropyridines, aflatoxin B1, and steroids [148].

TABLE 5.5 Percentage Amino Acid Homology for CYP2C Subfamily in Various Species

	Human CYP2C8	Human CYP2C9	Human CYP2C18	Human CYP2C19	Cyno-molgus Monkey CYP-2C20	Cyno-molgus Monkey CYP-2C43	Cyno CYP-2C75	Cyno CYP-2C76	Dog CYP-2c21	Dog CYP-2C41	Rat Cyp-2c6	Rat Cyp-2c7	Rat Cyp-2c11	Rat Cyp-2c12	Rat Cyp-2c13	Rat Cyp-2c23	Mouse Cyp-2c29	Mouse Cyp-2c37	Mouse Cyp-2c38	Mouse Cyp-2c39	Mouse Cyp-2c40
Human CYP2C8	100	77	77	77	92	78	76	70	66	70	71	68	73	64	64	60	71	68	71	71	65
Human CYP2C9	77	100	81	91	78	92	93	71	68	75	75	71	76	66	66	61	75	73	72	72	66
Human CYP2C18	77	81	100	81	76	80	81	71	69	75	77	71	77	67	68	61	76	73	74	74	66
Human CYP2C19	77	91	81	100	78	90	91	72	70	74	75	71	74	67	66	61	74	72	72	72	65
Cyno CYP2C20	92	78	76	78	100	77	76	69	67	70	71	68	74	66	65	60	71	69	71	71	65
Cyno CYP2C43	78	92	80	90	77	100	93	71	68	74	72	70	74	65	65	61	73	71	71	71	65
Cyno CYP2C75	76	93	81	91	76	93	100	70	69	74	74	71	74	65	66	61	75	71	72	72	65
Cyno CYP2C76	70	71	71	72	69	71	70	100	77	70	69	63	69	60	60	58	68	65	65	65	61
Dog CYP2c21	66	68	69	70	67	68	69	77	100	69	66	63	65	61	60	58	66	65	66	66	60
Dog CYP2C41	70	75	75	74	70	74	74	70	69	100	71	66	70	65	66	61	70	70	67	68	66
Rat Cyp2c6	71	75	77	75	71	72	74	69	66	71	100	75	72	67	69	59	82	76	77	79	67
Rat Cyp2c7	68	71	71	71	68	70	71	63	63	66	75	100	69	68	69	57	83	70	79	80	68
Rat Cyp2c11	73	76	77	74	74	74	74	69	65	70	72	69	100	65	65	60	73	71	71	71	64
Rat Cyp2c12	64	66	67	67	66	65	65	60	61	65	67	68	65	100	80	54	70	71	69	69	77
Rat Cyp2c13	64	66	68	66	65	65	66	60	60	66	69	69	65	80	100	54	70	72	70	70	79
Rat Cyp2c23	60	61	61	61	60	61	61	58	58	61	59	57	60	54	54	100	60	59	58	59	52
Mouse Cyp2c29	71	75	76	74	71	73	75	68	66	70	82	83	73	70	70	60	100	76	84	84	68
Mouse Cyp2c37	68	73	73	72	69	71	71	65	65	70	76	70	71	71	72	59	76	100	74	74	71
Mouse Cyp2c38	71	72	74	72	71	71	72	65	66	67	77	79	71	69	70	58	84	74	100	91	70
Mouse Cyp2c39	71	72	74	72	71	71	72	65	66	68	79	80	71	69	70	59	84	74	91	100	70
Mouse Cyp2c40	65	66	66	65	65	65	65	61	60	66	67	68	64	77	79	52	68	71	70	70	100

Rhesus monkey CYP2C43, CYP2C74, and CYP2C75 (not shown) display 99% amino acid homology to the cynomolgus monkey CYP2C43, CYP2C20, and CYP2C75 sequences, respectively.

Cyp2c11 (male), Cyp2c12 (female), and Cyp2c13 (male) are gender specific. The dog CYP2C21 and CYP2C41 enzymes are found in the liver, and the latter has been shown to be highly polymorphic. The substrate specificity (tolbutamide, warfarin, and *S*-mephenytoin) of the two dog CYP2C isoforms is impaired when compared to that of humans [149]. Monkey has genes encoding for CYP2C20 and CYP2C43, which are expressed in the liver. CYP2C43, but not CYP2C20, was able to metabolize *S*-mephenytoin, a probe of human CYP2C19, and was not able to metabolize tolbutamide, a probe substrate of human CYP2C9, suggesting that CYP2C43 appears to be functionally related to CYP2C19 [9,150]. Substrate specificities are largely different among the species, particularly, CYP2C forms in dog as a model for human. The stereoselective metabolism of mephenytoin, a marker substrate for CYP2C isoforms shows differences between species. In rabbit, dog, and rat, the R-enantiomer is preferentially 4′-hydroxylated two- to sixfold higher than that of the S-form. In female mice, both the enantiomers are equally good substrates; and in the rat, CYP2C11 preferentially metabolizes the S-enantiomer. However, both human and monkey CYP2C forms preferentially 4′-hydroxylate *S*-mephenytoin with similar K_m values, suggesting that monkeys may be useful as a human model in the metabolism of *S*-mephenytoin [151].

5.4.7 CYP2D

Amino acid homologies for the CYP2D subfamily are presented in Table 5.6. Human CYP2D6, the only active human CYP2D isoform, makes up approximately 1–3% of the total CYP content in the liver and catalyzes the biotransformation of ~30% clinically used drugs. CYP2D6 mainly catalyzes antidepressants (desipramine), β-blockers (propranolol), the antiarrhythmics (sparteine), and dextromethorphan, as well as methadone [152]. Using the dextromethorphan O-demethylation reaction as a selective marker of CYP2D activity [153,154], mouse, rat, rabbit, dog, and monkey microsomes displayed approximately 141%, 96%, 148%, 45%, and 234% of the human microsome activity, respectively [6].

SNPs have been extensively studied in the CYP2D6 enzyme and have been shown to cause a large degree of variability in drug turnover rates for CYP2D6 substrates [155]. CYP2D6 is also known to undergo gene deletion and multiplications in humans, a genetic process known as *copy number variation* (CNV), the result of which leads to variable enzyme expression and consequently altered activity [156]. The gene has been reported to be multiplied up to 12-fold in humans, resulting in an ultrarapid metabolizer phenotype [157]. Data on copy number variation of DMEs in preclinical species and what consequences these genetic differences may incur for extrapolations to humans is currently lacking, but it is known that CNVs exist in other species and it appears as though the mechanisms by which they occur are conserved [158]. This issue is further complicated by the fact that the well-documented CYP2D6 SNPs are carried over to the duplicated copies of the gene [159]. The ramifications of CNVs and the combination of CNVs and SNPs on extrapolating drug metabolism data from preclinical species to humans remain unclear and may be very difficult to assess due to conventional laboratory breeding techniques but it is worth noting.

There are as many as nine mouse Cyp2d isoforms but little is known about their catalytic functions. Rats have six Cyp2d isoforms expressed in various tissues, such as liver, kidney, and brain. Cyp2d1 is the likely rat orthologe of human CYP2D6 and shows a similar substrate specificity [160]. Dog CYP2D15 is the major CYP2D form

TABLE 5.6 Percentage Amino Acid Homology for CYP2D Subfamily in Various Species

	Human CYP2D6	Cyno-molgus Monkey CYP2D17	Rhesus Monkey	Dog CYP-2D15	Rat Cyp-2d1	Rat Cyp-2d3	Rat Cyp-2d4	Rat Cyp-2d10	Rat Cyp-2d18	Rat Cyp-2d26	Mouse Cyp2d9	Mouse Cyp2d10	Mouse Cyp2d11	Mouse Cyp2d26	Guine Pig Cyp2d16	Hamster Cyp2d20	Hamster Cyp2d27	Hamster Cyp2d28
Human CYP2D6	100	93	93	74	71	72	77	71	76	71	69	69	66	70	72	72	71	69
Cyno CYP2D17	93	100	98	76	71	72	76	71	76	72	70	70	67	72	72	73	73	70
Rhesus CYP2D42	93	98	100	75	71	72	76	71	76	72	70	71	67	71	72	74	72	70
Dog CYP2D15	74	76	75	100	65	67	70	64	70	66	63	64	61	68	68	67	66	67
Rat Cyp2d1	71	71	71	65	100	79	72	95	72	73	82	83	80	74	68	75	74	76
Rat Cyp2d3	72	72	72	67	79	100	75	79	75	77	78	79	75	78	70	77	76	76
Rat Cyp2d4	77	76	76	70	72	75	100	72	99	72	70	71	68	72	70	72	72	72
Rat Cyp2d10	71	71	71	64	95	79	72	100	73	72	81	83	79	73	67	75	74	76
Rat Cyp2d18	76	76	76	70	72	75	99	73	100	72	70	72	69	71	69	72	71	72
Rat Cyp2d26	71	72	72	66	73	77	72	72	72	100	72	73	70	88	71	82	81	72
Mouse Cyp2d9	69	70	70	63	82	78	70	81	70	72	100	87	85	75	68	73	72	75
Mouse Cyp2d10	69	70	71	64	83	79	71	83	72	73	87	100	87	74	67	75	73	77
Mouse Cyp2d11	66	67	67	61	80	75	68	79	69	70	85	87	100	71	64	72	71	73
Mouse Cyp2d26	70	72	71	68	74	78	72	73	71	88	75	74	71	100	70	79	79	73
Guinea Pig Cyp2d16	72	72	72	68	68	70	70	67	69	71	68	67	64	70	100	70	69	67
Hamster Cyp2d20	72	73	74	67	75	77	72	75	72	82	73	75	72	79	70	100	95	73
Hamster Cyp2d27	71	73	72	66	74	76	72	74	71	81	72	73	71	79	69	95	100	71
Hamster Cyp2d28	69	70	70	67	76	76	72	76	72	72	75	77	73	73	67	73	71	100

and also demonstrates enzymatic activities similar to human CYP2D6, such as 1′-hydroxylation of bufuralol and quinidine metabolism [7,98]. Cynomolgus monkey has CYP2D17 an isoform that is 93% identical to human CYP2D6 at the amino acid level. It metabolizes bufuralol and dextromethorphan and is strongly inhibited by quinidine. The marmoset monkey has two known isoforms of CYP2D: CYP2D30 and CYP2D19. Both enzymes have shown regioselectivity of debrisoquine hydroxylation at various positions. A comparison of the CYP2D marker activity, bufuralol 1′-hydroxylation, in liver microsomes of different species showed that rat, mouse, rabbit, and monkey CYP2D6 exhibit a K_m value about 10 times lower than that of human CYP2D6, whereas the V_{max} value is about 6–10 times higher than that of the human enzyme. The kinetic behavior of dog CYP2D15 and human CYP2D6 was shown to be very similar for the catalysis of bufuralol, metoprolol, and dextromethorphan [7].

5.4.8 CYP2E

CYP2E1 is the only member of the CYP2E subfamily (Table 5.7) and is expressed in many tissues such as the nose, oropharynx, lung, and liver. CYP2E1 plays a detoxification role in the metabolism of alcohol to reduce alcohol levels after excessive intake and is also involved in the metabolism of ketones and fatty acids associated with diabetes and obesity [161]. CYP2E1 metabolizes small molecule drugs such as APAP, enflurane, caffeine, and chlorzoxazone (a marker substrate) [162]. In addition to these drugs, some environmental carcinogens and toxicants (benzene, styrene, acrylonitrile, and nitrosoamines) are also metabolized by CYP2E1 to superoxide radicals that cause liver injury and bind to DNA or cellular proteins [163,164]. CYP2E1 is inducible but the mechanism differs from the increased mRNA transcription that occurs after activation of the xenobiotic receptors AHR, CAR, and PXR. Instead, this inducibility occurs through increased translational efficiency and decreased protein turnover [165,166]. CYP2E1 induction by alcohol has been demonstrated in both animals [167] and man [168].

CYP2E1 is known to be highly conserved in mammals [169], and the metabolic pattern of CYP2E1 in humans appears to be very similar to that observed in rodents, and therefore, rodents and rat, in particular, may be an appropriate model to study CYP2E1 in man [98]. In further support of the hypothesis that there is a high level of functional conservation of CYP2E1 across species, it has been shown that in a chlorzoxazone 6-hydroxylase (6-OH-CLZ) assay [170], CYP2E1 activity in mouse, rat, rabbit, dog, and monkey was 150%, 47%, 45%, 45%, and 78% of the average human activity, respectively [6]. This result demonstrates that there is much less variability in chlorzoxazone metabolism than what is generally seen for marker substrates of the other CYP subfamilies. Although chlorzoxazone is considered to be a very good marker substrate for CYP2E1 in mouse, rat, rabbit, dog, monkey, and man, other enzymes have been implicated in its metabolism. Dog CYP1A has been shown to be catalytically active in the metabolism of chlorzoxazone, and human and rodent CYP1A2 may also contribute to chlorzoxazone metabolism [7].

5.4.9 CYP3A

The CYP3A subfamily (Table 5.8) plays a very important role in the metabolism of xenobiotics and catalyzes more than 50% of therapeutic drugs [171], such as

TABLE 5.7 Percentage Amino Acid Homology for CYP2E Subfamily in Various Species

	Human CYP2E1	Cynomolgus Monkey CYP2E1	Rhesus Monkey CYP2E1	Dog CYP2E1	Rat Cyp2e1	Mouse Cyp2e1	Rabbit Cyp2e1	Hamster Cyp2e1
Human CYP2E1	100	91	94	77	78	78	79	78
Cyno CYP2E1	91	100	95	73	77	76	76	75
Rhesus CYP2E1	94	95	100	76	80	79	79	79
Dog CYP2E1	77	73	76	100	76	76	75	76
Rat Cyp2e1	78	77	80	76	100	93	81	90
Mouse Cyp2e1	78	76	79	76	93	100	80	88
Rabbit Cyp2e1	79	76	79	75	81	80	100	79
Hamster Cyp2e1	78	75	79	76	90	88	79	100

TABLE 5.8 Percentage Amino Acid Homology for CYP3A Subfamily in Various Species

	Human CYP3A4	Human CYP3A5	Human CYP3A7	Human CYP3A43	Cynomolgus Monkey CYP3A8	Dog CYP-3A12	Rat Cyp-3a1	Rat Cyp-3a2	Rat Cyp-3a9	Rat Cyp-3a18	Mouse Cyp-3a11	Mouse Cyp-3a13	Mouse Cyp-3a16	Mouse Cyp-3a25	Mouse Cyp-3a41	Rabbit Cyp-3a6	Guinea Pig Cyp3a14	Guinea Pig Cyp3a15	Guinea Pig Cyp3a17	Hamster Cyp3a10	Hamster Cyp3a31
Human CYP3A4	100	84	88	75	93	79	73	72	76	68	72	75	70	71	71	75	71	71	70	67	69
Human CYP3A5	84	100	81	75	83	78	72	70	75	68	73	74	70	71	72	74	70	69	69	67	68
Human CYP3A7	88	81	100	71	89	74	70	69	72	65	70	72	68	67	68	72	69	68	67	64	66
Human CYP3A43	75	75	71	100	73	72	63	63	67	64	64	67	63	66	63	70	64	63	63	61	62
Cyno CYP3A8	93	83	89	73	100	77	71	71	75	67	71	74	69	69	70	74	71	69	69	66	68
Dog CYP3A12	79	78	74	72	77	100	69	69	75	66	70	73	67	68	69	74	68	67	66	66	66
Rat Cyp3a1	73	72	70	63	71	69	100	88	72	69	87	70	83	70	86	70	70	69	69	68	81
Rat Cyp3a2	72	70	69	63	71	69	88	100	71	67	85	69	81	67	83	68	68	67	67	66	81
Rat Cyp3a9	76	75	72	67	75	75	72	71	100	68	73	91	69	71	71	74	70	69	69	68	70
Rat Cyp3a18	68	68	65	64	67	66	69	67	68	100	69	67	66	90	67	64	67	66	65	79	65
Mouse Cyp3a11	72	73	70	64	71	70	87	85	73	69	100	70	87	70	91	71	70	68	68	69	81
Mouse Cyp3a13	75	74	72	67	74	73	70	69	91	67	70	100	66	69	67	72	68	68	67	67	68
Mouse Cyp3a16	70	70	68	63	69	67	83	81	69	66	87	66	100	67	87	68	67	66	66	66	76
Mouse Cyp3a25	71	71	67	66	69	68	70	67	71	90	70	69	67	100	69	66	69	68	67	80	66
Mouse Cyp3a41	71	72	68	63	70	69	86	83	71	67	91	67	87	69	100	70	68	66	67	67	78
Rabbit Cyp3a6	75	74	72	70	74	74	70	68	74	64	71	72	68	66	70	100	71	69	68	65	68
Guinea Pig Cyp3a14	71	70	69	64	71	68	70	68	70	67	70	68	67	69	68	71	100	96	93	66	69
Guinea Pig Cyp3a15	71	69	68	63	69	67	69	67	69	66	68	68	66	68	66	69	96	100	92	66	67
Guinea Pig Cyp3a17	70	69	67	63	69	66	69	67	69	65	68	67	66	67	67	68	93	92	100	66	68
Hamster Cyp3a10	67	67	64	61	66	66	68	66	68	79	69	67	66	80	67	65	66	66	66	100	66
Hamster Cyp3a31	69	68	66	62	68	66	81	81	70	65	81	68	76	66	78	68	69	67	68	66	100

terfenadine, midazolam, diazepam, quinidine, lidocaine, carbamazepine, nifedipine, tacrolimus, dapsone, erythromycin, and dextromethorphan [172]. In addition, CYP3A is also involved in the metabolism of endogenous substances such as steroids, bile acids, and retinoic acid. Human CYP3A enzymes (CYP3A4 and CYP3A5) are expressed in liver, stomach, lung, intestine, and kidney and are the most abundant CYP isoforms in human liver [173]. Using midazolam 1′-hydroxylase (1′-OH-MDZ) as a measure of CYP3A activity [174], mouse, rat, rabbit, dog, and monkey microsomal levels of activity were found to be approximately 103%, 9.2%, 20%, 137%, and 128%, respectively, of the average human activity [6]. Midazolam is a marker substrate of human CYP3A4 and shows significant interspecies variability in metabolism. Higher rates of midazolam 1′-hydroxylation were observed in dogs and monkeys in comparison with human; however, no significant differences were observed between cynomolgus and rhesus monkeys [175].

Mouse has at least six Cyp3a isoforms of which Cyp3a11 is believed to be the orthologe of human CYP3A4. The mouse Cyp3a41 and Cyp3a42 isoforms are preferentially expressed in females [176]. The catalytic activity of the mouse CYP3A toward clinically used drugs has not been extensively evaluated, but it has been shown that some chemicals that are metabolized by human CYP3A4 can be also catalyzed by mouse CYP3A, such as aflatoxin B1 and ethylmorphine [177]. Rats have six Cyp3a isoforms that are expressed in a gender-specific manner. For example, Cyp3a2 and Cyp3a18 are male specific, whereas Cyp3a9 is a female-dominant isoform [178]. Oral bioavailability does not generally correlate well between human and rat forms, and this may be due to higher Cyp3a expression in rat intestine and consequently increased first-pass metabolism [5]. Rats are also not an appropriate model for human CYP3A induction because of the lack of induction response of Cyp3a1/2 to rifampin, a known inducer of human CYP3A [179]. In addition, the metabolism of some CYP3A substrates in rats is different from that in human. For example, nifedipine and dihydropyridine are not metabolized by rat CYP3A [4,10]. The dog CYP3A family has two isoforms, CYP3A12 and CYP3A26. Dog CYP3A has shown similar catalytic activities toward the marker substrates for human CYP3A4 [129,180]. Cynomolgus monkey CYP3A8 is expressed largely in the liver and shows 93% amino acid sequence homology to human CYP3A4 and is identical to rhesus monkey CYP3A64 [181]. CYP3A64 is capable of metabolizing testosterone, midazolam, nifedipine, and 7-benzoxy-4-trifluoromethylcoumarin, which are also substrates for human CYP3A4 and CYP3A5. However, species differences in the CYP3A catalytic activities are observed among rats, rabbits, dogs, and humans.

Testosterone 6β-hydroxylation is a commonly used marker activity for CYP3A enzymes in all species. There is no significant difference in the reaction among the species, with exception of dogs (higher K_m value) and female rats (higher K_m and lower V_{max} values) [7]. Rats demonstrate a sex-related difference in CYP3A activity. Rat CYP3A not only shows higher rates of the 6β-hydroxylation than human CYP3A but also generates 2β- and 16β-hydroxyltestosterone. On the basis of kinetic similarity in liver microsomes for testosterone 6β-hydroxylation, mouse and male rat appear to be the most similar species to human. Mouse microsomal testosterone 6β-hydroxylation is higher in females than in males probably due to the catalytic activity of CYP3A41, a female-specific form [182]. Lidocaine metabolism provides another strong example of interspecies differences in CYP3A metabolism. In humans, lidocaine undergoes an N-deethylation catalyzed by CYP3A4 [183,184]; however, in rats, the major metabolite

is formed through 3-hydroxylation catalyzed by a member of the CYP2D subfamily [1,185]. In general, CYP3A enzymes in all species are involved in the metabolism of xenobiotics and show species-related differences in substrate specificity, expression, induction, and inhibition in various organs and tissues, which together contribute to altered drug clearance, efficacy, and toxicity across species. This wide degree of interspecies variation makes the extrapolation of CYP3A data from animals to man quite difficult.

5.5 GLUCURONOSYLTRANSFERASES (UGTs)

The UGTs catalyze the addition of a glucuronic acid moiety to nucleophilic substrates using uridine diphosphate/glucuronic acid (UDPGA) as a cofactor. The UGT superfamily is composed of a large multiplicity of forms, each with a broad and overlapping substrate selectivity and wide tissue distribution [186,187]. Three major subfamilies responsible for drug metabolism are present to date: UGT1A (Table 5.9), UGT2B (Table 5.10), and UGT2A (Table 5.11). Similar to CYPs, UGTs are localized in the endoplasmic reticulum membrane; however, the active site is exposed on the lumenal side of the endoplasmic reticulum, while CYPs are on the cytosolic side [188]. Many endogenous and xenobiotic compounds are able to be glucuronidated by the UGTs. For example, bilirubin and estradiol-3-glucuronidations are selective for UGT1A1, propofol is glucuronidated by UGT1A9, and AZT and morphine glucuronidations occur through UGT2B7. Other endogenous substrates that undergo glucuronidation include bilirubin, estradiol, and other steroids, as well as some fatty acids [189]. UGT1A3 and UGT1A4 are expressed in the liver, intestine, and colon and are the important enzymes in the glucuronidation of many tertiary amine or aromatic heterocycles to form quaternary ammonium glucuronides. The formation of quaternary ammonium glucuronides appears be highly species specific, with the highest activity being found in humans and monkeys. Rats and mice are generally incapable of forming quaternary ammonium glucuronides because the UGT1A3 and UGT1A4 isoforms are pseudogenes in these rodents. Lamotrigine is extensively glucuronidated at the 2-position of the triazine ring in humans, which represents $>80\%$ of the dose excreted in urine. Lamotrigine is not significantly glucuronidated in rats and dogs, while 60% of the dose is excreted in guinea pig urine as the 2-*N*-glucuronide [190]. UGT1A6 is the most important enzyme for the conjugation of planar phenols and amines. It displays high activity for a variety of aromatic alcohols, such as 1-naphthol, 4-nitrophenol, 4-methylumbelliferone, and in the detoxification of APAP. Cats are highly susceptible to APAP liver toxicity because UGT1A6 is a pseudogene in this species [191]. Afloqualone (AFQ) is a centrally acting muscle relaxant, and AFQ *N*-glucuronide is the most abundant metabolite in human urine when administered orally, whereas it was not detected in urine when administered to rats, dogs, and monkeys [192]. Species differences in AFQ N-glucuronidation were investigated with liver microsomes obtained from humans and experimental animals. The K_m and V_{max} values of glucuronidation in HLMs were 2.0 mM and 0.87 nmol/min/mg protein, respectively. The V_{max} and intrinsic clearance (CL_{int}) values of AFQ N-glucuronidation in human liver were approximately 4- to 10-fold and 2- to 4-fold higher, respectively, than those in rat, dog, and monkey. Both recombinant UGT1A4 and UGT1A3 exhibited high AFQ *N*-glucuronosyltransferase activities.

TABLE 5.9 Percentage Amino Acid Homology for UGT1A Subfamily in Various Species

	Human UGT-1A1	Human UGT-1A3	Human UGT-1A4	Human UGT-1A5	Human UGT-1A6	Human UGT-1A7	Human UGT-1A8	Human UGT-1A9	Human UGT-1A10	Cyno-molgus Monkey UGT1A01	Cyno-molgus Monkey UGT1A06	Cyno-molgus Monkey UGT1A08	Dog UGT-1A6	Rat Ugt-1a1	Rat Ugt-1a2	Rat Ugt-1a3	Rat Ugt-1a6	Rat Ugt-1a8	Mouse Ugt1a1	Mouse Ugt1a2	Mouse Ugt1a6	Mouse Ugt1a9
Human UGT1A1	100	71	71	72	67	66	65	66	65	95	66	64	61	78	66	67	62	61	77	66	61	62
Human UGT1A3	71	100	93	94	66	66	67	67	66	69	65	65	60	64	75	75	61	60	64	75	60	62
Human UGT1A4	71	93	100	93	66	66	66	67	66	69	65	65	61	65	74	74	62	61	64	74	61	61
Human UGT1A5	72	94	93	100	66	67	67	67	66	70	64	66	60	64	75	74	61	60	64	74	61	62
Human UGT1A6	67	66	66	66	100	68	68	68	68	66	95	66	80	62	62	63	79	62	62	63	79	63
Human UGT1A7	66	66	66	67	68	100	94	93	93	65	66	92	61	62	61	60	62	77	62	62	63	78
Human UGT1A8	65	67	66	67	68	94	100	94	94	64	65	95	61	62	62	60	62	78	63	62	63	78
Human UGT1A9	66	67	67	67	68	93	94	100	93	65	66	93	61	63	63	61	63	78	63	62	64	78
Human UGT1A10	65	66	66	66	68	93	94	93	100	65	65	92	61	62	62	60	61	77	62	62	62	78
Cyno UGT1A01	95	69	69	70	66	65	64	65	65	100	68	66	62	78	66	67	62	61	78	66	62	62
Cyno UGT1A06	66	65	65	64	95	66	65	66	65	68	100	67	80	62	61	63	80	61	62	63	79	62
Cyno UGT1A08	64	65	65	66	66	92	95	93	92	66	67	100	62	62	62	60	61	78	63	62	62	79
Dog UGT1A6	61	60	61	60	80	61	61	61	61	62	80	62	100	63	61	63	78	60	62	61	78	60
Rat Ugt1a1	78	64	65	64	62	62	62	63	62	78	62	62	63	100	69	70	66	67	91	68	65	67
Rat Ugt1a2	66	75	74	75	62	61	62	63	62	66	61	62	61	69	100	83	66	66	69	91	65	66
Rat Ugt1a3	67	75	74	74	63	60	60	61	60	67	63	60	63	70	83	100	68	65	70	80	66	64
Rat Ugt1a6	62	61	62	61	79	62	62	63	61	62	80	61	78	66	66	68	100	66	66	65	93	65
Rat Ugt1a8	61	60	61	60	62	77	78	78	77	61	61	78	60	67	66	65	66	100	65	65	65	84
Mouse Ugt1a1	77	64	64	64	62	62	63	63	62	78	62	63	62	91	69	70	66	65	100	68	64	66
Mouse Ugt1a2	66	75	74	74	63	62	62	62	62	66	63	62	61	68	91	80	65	65	68	100	65	66
Mouse Ugt1a6	61	60	61	61	79	63	63	64	62	62	79	62	78	65	65	66	93	65	64	65	100	66
Mouse Ugt1a9	62	62	61	62	63	78	78	78	78	62	62	79	60	67	66	64	65	84	66	66	66	100

TABLE 5.10 Percentage Amino Acid Homology for UGT2A Subfamily in Various Species

	Human UGT2A1	Human UGT2A3	Rat Ugt2a1	Mouse Ugt2a1	Mouse Ugt2a3	Guinea Pig Ugt2a3
Human UGT2A1	100	61	87	88	72	65
Human UGT2A3	61	100	60	60	62	71
Rat Ugt2a1	87	60	100	95	76	65
Mouse Ugt2a1	88	60	95	100	78	64
Mouse Ugt2a3	72	62	76	78	100	66
Guinea Pig Ugt2a3	65	71	65	64	66	100

Expression of the UGT1A gene utilizes different promoters that yield transcripts containing alternative first exons and four identical downstream exons [193]. There is a 93–94% sequence homology in the first exon of human UGT1A7 to UGT1A10, two isoforms that show considerable variation in the level of tissue expression. UGT1A9 is expressed in human liver and kidney, whereas UGT1A7 (esophagus and gastric epithelium), UGT1A8 (intestine), and UGT1A10 (intestine) are expressed extrahepatically. In contrast, rat and rabbit Ugt1a7 are expressed in liver. The rabbit UGT1A7 shows high activity for a variety of small phenolic molecules and imipramine. Rat UGT1A7 catalyzes the glucuronidation of benzo[*a*]pyrene phenols and is inducible by 3-methylcholanthrene. Human UGT1A7 has high turnover for the glucuronidation of 7-ethyl-10-hydroxycamptothecin, an active metabolite of irinotecan, and therefore, may play a role in the gastrointestinal first-pass metabolism of the drug along with UGT1A8 and UGT1A10 [194]. UGT1A8 catalyzes reactions of several drugs including, opioids (buprenorphine, morphine, naloxone, and naltrexone), ciprofibrate, diflunisal, furosemide, phenolphthalein, propofol, raloxifene, 4-OH-tamoxifen, and tolcapone [194]. UGT1A9 is largely responsible for the glucuronidation of a variety of bulky phenols, for example, *tert*-butylphenols and propofol (a marker substrate for UGT1A8 and UGT1A9). UGT2B7 is expressed in the liver and involved in the glucuronidation of non-steroidal anti-inflammatory drugs (NSAIDs), morphine, 3-OH-benzodiazepines, and zidovudine. The glucuronidation of morphine to a pharmacologically active metabolite by human UGT2B7 has been well studied. Morphine-6-glucuronide is much more potent in receptor binding than morphine; however, it has poor permeability and is unlikely to cross the blood–brain barrier. Rats are unable to make morphine-6-glucuronide. UGT2B15 and UGT2B17 are the major forms in human prostate and other tissues such as liver, kidney, skin, brain, mammary gland, ovaries, adipose tissue, and uterus and are responsible for the conjugation of androgens and drugs such as racemic oxazepam [195]. Little has been reported on the species difference of these conjugation reactions.

5.6 SULFOTRANSFERASES (SULTs)

The cytosolic SULTs are derived from a large gene superfamily. SULT1 and SULT2 families are the most important for xenobiotic metabolism. SULTs catalyze the sulfation reactions of many endogenous substances and drugs in the presence of PAPS

TABLE 5.11 Percentage Amino Acid Homology for UGT2B Subfamily in Various Species

	Human UGT-2B4	Human UGT-2B7	Human UGT-2B10	Human UGT-2B11	Human UGT-2B15	Human UGT-2B17	Cyno-molgus Monkey UGT2B9	Cyno-molgus Monkey UGT2B18	Cyno-molgus Monkey UGT2B19	Cyno-molgus Monkey UGT2B20	Cyno-molgus Monkey UGT2B23	Cyno-molgus Monkey UGT2B30	Dog UGT-2B31	Rat Ugt-2b1	Rat Ugt-2b2	Rat Ugt-2b3	Rat Ugt-2b4	Rat Ugt-2b5	Mouse Ugt-2b36	Mouse Ugt-2b37	Mouse Ugt-2b34
Human UGT2B4	100	85	85	85	78	78	85	86	88	76	83	88	77	71	65	65	66	64	68	66	70
Human UGT2B7	85	100	87	85	78	76	89	89	82	75	87	82	75	69	65	65	67	64	67	64	68
Human UGT2B10	85	87	100	90	77	77	87	87	81	75	84	80	74	67	62	62	64	62	62	67	68
Human UGT2B11	85	85	90	100	76	76	86	86	81	74	83	81	73	68	61	62	64	61	65	63	66
Human UGT2B15	78	78	77	76	100	94	78	78	76	92	77	77	76	71	65	65	67	65	69	66	67
Human UGT2B17	78	76	77	76	94	100	77	77	76	90	75	77	76	70	65	66	68	66	69	67	67
Cyno UGT2B9	85	89	87	86	78	77	100	95	82	75	94	82	75	68	64	64	66	63	66	65	69
Cyno UGT2B18	86	89	87	86	78	77	95	100	82	75	94	82	75	70	64	64	66	63	66	64	69
Cyno UGT2B19	88	82	81	81	76	76	82	82	100	75	80	94	75	68	63	63	66	63	67	64	67
Cyno UGT2B20	76	75	75	74	92	90	75	75	75	100	74	75	75	69	65	65	67	64	68	67	67
Cyno UGT2B23	83	87	84	83	77	75	94	94	80	74	100	79	73	67	62	62	65	62	65	63	67
Cyno UGT2B30	88	82	80	81	77	77	82	82	94	75	79	100	75	67	63	64	66	63	67	65	68
Dog UGT2B31	77	75	74	73	76	76	75	75	75	75	73	75	100	73	64	64	66	64	68	65	70

Rat Ugt2b1	71	69	67	68	71	70	68	70	68	69	67	67	73	100	61	61	63	60	65	61	68
Rat Ugt2b2	65	65	62	61	65	65	64	64	63	65	62	63	64	61	100	84	77	83	79	81	60
Rat Ugt2b3	65	65	62	62	65	66	64	64	63	65	62	64	64	61	84	100	79	92	81	85	62
Rat Ugt2b4	66	67	64	64	67	68	66	66	66	67	65	66	66	63	77	79	100	78	82	78	63
Rat Ugt2b5	64	64	62	61	65	66	63	63	63	64	62	63	64	60	83	92	78	100	80	85	62
Mouse Ugt2b36	68	67	62	65	69	69	66	66	67	68	65	67	68	65	79	81	82	80	100	82	63
Mouse Ugt2b37	66	64	67	63	66	67	65	64	64	67	63	65	65	61	81	85	78	85	82	100	61
Mouse Ugt2b34	70	68	68	66	67	67	69	69	67	67	67	68	70	68	60	62	63	62	63	61	100

(3′-phosphoadnosine 5′-phosphosulfate), providing the sulfuryl donor for the reactions. In humans, the SULT enzyme family comprises 11 isoforms of the SULT1, SULT2, and SULT4 families that code for 10 distinct genes. In humans, SULT1A1 is expressed in the liver and metabolizes sulfation of many known drugs, such as APAP, troglitazone, minoxidil, 4-OH-tamoxifen, and apomorphine. SULT1A3, a catecholamine sulfotransferase, is absent in the adult liver, with the major site of expression being the gastrointestinal tract. This has implications for the metabolism of a number of drugs; for example, salbutamol is almost exclusively sulfated in humans. Human SULT1C2 and SULT1C4 are almost exclusively expressed in fetal tissues, although the function of these enzymes remains unclear. Another important enzyme is estrogen sulfotransferase (SULT1E1), which has a very high affinity (low nanomolar) for its natural substrate 17β-estradiol and for a major drug substrate 17α-ethinylestradiol. SULT1E1 is important for modulating estrogen action in the endometrium [196,197]. SULT2A1 plays a selective role in the N-sulfation of quinolone drugs and other amine drugs (ciprofloxacin, moxifloxacin, garenoxacin, desipramine), as well as metoclopramide, pregnenolone, and dehydroepiandrosterone [198]. SULT4A1 cDNAs have been isolated so far from rats, mice. and humans, and the predicted proteins share a remarkable degree of similarity. The SULT4A1 proteins seem to be expressed only in the brain, and despite significant efforts, no natural or xenobiotic substrate has yet been identified [199,200]. Mouse Sult6b1 has been recently cloned and expressed, and it exhibited sulfation activity toward thyroxine and bithionol and a variety of other endogenous and xenobiotic compounds. While human SULT6B1 was specifically expressed in kidney and testis, mouse Sult6b1 was detected in brain, heart, kidney, thymus, lung, liver, and testis [201].

5.7 FLAVIN-CONTAINING MONOOXYGENASES (FMOs)

FMOs are a family of FAD-containing enzymes located in the endoplasmic reticulum. At least five gene products are known to exist in mammals (Table 5.12). The catalytic function of these enzymes involves the oxygenation of soft nucleophilic heteroatoms, including nitrogen, sulfur, phosphorus, and selenium. FMOs catalyze the biotransformation of a wide variety of xenobiotics and endogenous compounds. Human FMO1 has the broadest substrate specificity, particularly for bulky nonpolar lipophilic compounds such as imipramine and orphenadrine, which are selective markers for FMO1 in the kidney. Human FMO3, present in the liver, has a unique property to selectively metabolize small amines, such as trimethylamine. FMO5 is expressed in adult liver and has poor catalytic activity compared to other FMOs. It stereoselectively catalyzes sulfoxidation of short-chain arylalkyl sulfides. Little is reported about the species difference of their catalytic activity. However, the tissue distribution of FMOs in animal species has been reported in rabbits [202]. Rabbit Fmo1 mRNA expression is highest in liver and intestine, Fmo2 in lung, while Fmo3, Fmo4, and Fmo5 are present in lung and kidney. FMO1 seems to be the dominant form in pig and male rat livers. Hepatic Fmo3 expression levels in adult rat and rabbit livers show no gender difference but there is an age-dependent change. In dogs, FMO1 and FMO3 are expressed in liver and lung, with small amounts of FMO3 expression in kidney. There is also a significant gender difference in the hepatic expression of FMO isoforms that exist in mice.

TABLE 5.12 Percentage Amino Acid Homology for FMO Family in Various Species

	Human FMO1	Human FMO2	Human FMO3	Human FMO4	Human FMO5	Dog FMO1	Dog FMO3	Rat FMO1	Rat FMO2	Rat FMO3	Rat FMO4	Rat FMO5	Mouse FMO1	Mouse FMO2	Mouse FMO3	Mouse FMO4	Mouse FMO5	Rabbit FMO1	Rabbit FMO2	Rabbit FMO3	Rabbit FMO4	Rabbit FMO5	Guinea Pig FMO2	Guinea Pig FMO5
Human FMO1	100	57	54	50	51	88	55	82	56	53	51	50	83	56	53	51	50	86	58	54	51	51	56	50
Human FMO2	57	100	57	53	55	56	57	56	84	57	53	55	55	85	56	52	55	55	86	56	53	55	85	55
Human FMO3	54	57	100	52	52	55	83	54	56	80	52	52	54	56	79	51	53	54	57	83	53	53	56	54
Human FMO4	50	53	52	100	51	51	53	49	52	52	80	51	50	53	51	79	50	52	52	52	83	51	52	51
Human FMO5	51	55	52	51	100	51	51	50	56	50	48	84	49	56	51	48	84	51	56	51	49	84	55	87
Dog FMO1	88	56	55	51	51	100	56	84	56	54	52	50	84	56	55	51	50	87	57	55	51	51	56	51
Dog FMO3	55	57	83	53	51	56	100	55	56	81	52	53	55	56	80	51	53	55	58	84	54	53	57	54
Rat FMO1	82	56	54	49	50	84	55	100	56	53	50	50	94	55	53	49	50	83	56	54	49	50	54	51
Rat FMO2	56	84	56	52	56	56	56	56	100	56	53	56	55	94	56	52	55	55	86	55	53	55	83	56
Rat FMO3	53	57	80	52	50	54	81	53	56	100	51	52	53	57	90	50	52	53	57	84	52	52	57	52
Rat FMO4	51	53	52	80	48	52	52	50	53	51	100	49	50	54	51	90	49	51	53	51	78	48	51	50
Rat FMO5	50	55	52	51	84	50	53	50	56	52	49	100	49	55	52	49	92	50	55	53	51	83	55	83
Mouse FMO1	83	55	54	50	49	84	55	94	55	53	50	49	100	55	54	49	49	83	56	55	48	50	53	50
Mouse FMO2	56	85	56	53	56	56	56	55	94	57	54	55	55	100	56	52	55	55	87	55	53	56	84	56
Mouse FMO3	53	56	79	51	51	55	80	53	56	90	51	52	54	56	100	50	53	54	56	82	51	53	56	54
Mouse FMO4	51	52	51	79	48	51	51	49	52	50	90	49	49	52	50	100	49	50	52	50	77	48	51	49
Mouse FMO5	50	55	53	50	84	50	53	50	55	52	49	92	49	55	53	49	100	49	55	53	51	83	54	84
Rabbit FMO1	86	55	54	52	51	87	55	83	55	53	51	50	83	55	54	50	49	100	56	54	51	50	54	51
Rabbit FMO2	58	86	57	52	56	57	58	56	86	57	53	55	56	87	56	52	55	56	100	56	53	55	85	56
Rabbit FMO3	54	56	83	52	51	55	84	54	55	84	51	53	55	55	82	50	53	54	56	100	53	54	56	53
Rabbit FMO4	51	53	53	83	49	51	54	49	53	52	78	51	48	53	51	77	51	51	53	53	100	50	52	51
Rabbit FMO5	51	55	53	51	84	51	53	50	55	52	48	83	50	56	53	48	83	50	55	54	50	100	55	82
Guinea Pig FMO2	56	85	56	52	55	56	57	54	83	57	51	55	53	84	56	51	54	54	85	56	52	55	100	56
Guinea Pig FMO5	50	55	54	51	87	51	54	51	56	52	50	83	50	56	54	49	84	51	56	53	51	82	56	100

5.8 SUMMARY

In the pharmaceutical industry, the investigation of drug metabolism catalyzed by DMEs in various species is essential for drug development and helps us to understand whether the drug candidates will behave well in humans by demonstrating sufficient efficacy and little or no toxicological liability. Although it is generally believed that data from animal studies can be extrapolated to humans reasonably well with the application of appropriate pharmacokinetic principles, there are certainly some limitations. *In vitro* studies of the DMEs, such as reaction phenotyping, enzyme kinetics, induction, and inhibition, can provide information on the underlying mechanisms and possible prediction of the species differences between drugs and drug candidates in pharmacokinetics, DDIs, efficacy, and toxicity. In addition, the expression level of the enzymes among species responsible for the biotransformation of test drugs is also critical because the metabolic clearance of a drug in the body contributes not only to the catalytic activity of the enzyme(s) but also to the total concentration of the enzyme(s) responsible for the clearance. Therefore, species differences in drug metabolism are attributed to specific activity (product formed per unit time per unit enzyme) for a drug, expression level of the metabolizing enzyme(s), and, in some cases, differences between the inducible expression of the responsible enzyme(s).

The selection of the best animal model to be used in development of a new drug is difficult. Caution should be taken in extrapolation of animal data to humans; however, when *in vitro* metabolism studies with liver microsomes, hepatocytes, liver slices, and recombinant enzymes are available, the information can be incorporated into the decision-making process and can aid in the selection of the appropriate *in vivo* animal model. It is important to remember that it is highly unlikely that any animal model can appropriately model all aspects of drug metabolism for a given NCE, and in many cases, different species may be needed to answer specific questions. Further, as more and more *in vitro* tools are developed, more of these questions are likely to be answered without the use of *in vivo* models, further decreasing the number of animals used in the early drug development process.

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