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Mammalian flavin-containing monooxygenases: structure/function, genetic polymorphisms and role in drug metabolism

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Abstract

Flavin-containing monooxygenase (FMO) oxygenates drugs and xenobiotics containing a "soft-nucleophile", usually nitrogen or sulfur. FMO, like cytochrome *P*450 (CYP), is a monooxygenase, utilizing the reducing equivalents of NADPH to reduce 1 atom of molecular oxygen to water, while the other atom is used to oxidize the substrate. FMO and CYP also exhibit similar tissue and cellular location, molecular weight, substrate specificity, and exist as multiple enzymes under developmental control. The human FMO functional gene family is much smaller (5 families each with a single member) than CYP. FMO does not require a reductase to transfer electrons from NADPH and the catalytic cycle of the 2 monooxygenases is strikingly different. Another distinction is the lack of induction of FMOs by xenobiotics.

In general, CYP is the major contributor to oxidative xenobiotic metabolism. However, FMO activity may be of significance in a number of cases and should not be overlooked. FMO and CYP have overlapping substrate specificities, but often yield distinct metabolites with potentially significant toxicological/pharmacological consequences.

The physiological function(s) of FMO are poorly understood. Three of the 5 expressed human FMO genes, FMO1, FMO2 and FMO3, exhibit genetic polymorphisms. The most studied of these is FMO3 (adult human liver) in which mutant alleles contribute to the disease known as trimethylaminuria. The consequences of these FMO genetic polymorphisms in drug metabolism and human health are areas of research requiring further exploration.

Keywords

Flavin monooxygenase; Drug metabolism; FMO

Abbreviations

BVMOs, Baeyer–Villiger monooxygenases; CYP, cytochrome P450; DBM, dinucleotide-binding motif; FADPNR, FAD-dependent pyridine nucleotide reductase PRINTS signature; FMO, flavin-containing monooxygenase; FMOXYGENASE, FMO PRINTS signature; GR, glutathione reductase; PAMO, phenylacetone monooxygenase; PNDRDTASEI, pyridine nucleotide disulfide reductase class-I PRINTS signature; ROS, reactive oxygen species; SNP, single-nucleotide polymorphism; TMAU, trimethylaminuria

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1. Introduction

Mammalian flavin-containing monooxygenase (FMO, EC 1.14.13.8) was first described by Dr. Daniel Ziegler and colleagues at the University of Texas at Austin (reviewed in Ziegler, 1980, 1988, 1991, 1993, 2002; Poulsen & Ziegler, 1995). This hepatic microsomal enzyme system was demonstrated to utilize oxygen and NADPH to convert *N*,*N*′-dimethylaniline to the N-oxide (Ziegler & Pettit, 1964; Mitchell & Zieger, 1969). The location, co-factor requirement and activity of FMO toward xenobiotics were very similar to the then recently characterized cytochrome *P*450 (CYP) monooxygenase and for a time this enzyme was known as "Ziegler's enzyme". In 1971, the enzyme was purified from porcine liver microsomes and definitively demonstrated to be a distinct monooxygenase containing flavin, with no heme (Ziegler et al., 1971a; Ziegler & Mitchell, 1972; Ziegler & Poulsen, 1978). Again, based on studies on the catalytic activity of this enzyme, it was referred to as "mixed-function amine oxidase", a term that did not capture its wide substrate range.

FMO is a flavoprotein containing a single FAD. Much of the early work describing the structure/function, mechanism, regulation, and substrate specificity of FMO was with the purified porcine liver enzyme, again led by Dr. Dan Ziegler and his colleague Dr. Larry Poulsen (Ziegler et al., 1971a, 1971b, 1992; Poulsen & Ziegler, 1979, 1995). The pig liver enzyme oxygenates a wide range of sulfur- and nitrogen-containing xenobiotics and, in some cases, also oxygenates selenium, iodine, boron, phosphorus and even carbon. An unusual feature of this class of enzymes is that substrate binding has no effect on velocity. In general, any chemical containing a soft nucleophile that gains access to the peroxyflavin intermediate (see Section 2.1 for details) is a potential substrate.

In 1984, the first direct evidence of the existence of multiple forms of FMO was obtained when the enzyme was purified from rabbit lung microsomes independently by 2 laboratories (Williams et al., 1984; Tynes et al., 1985). The "lung" FMO was distinct from the "liver" FMO in having high activity toward primary alkyl amines, restricted substrate specificity related to steric properties, resistance to detergent inhibition and enhanced thermal stability (Williams et al., 1984, 1985, 1990; Tynes&Hodgson, 1985a, 1985b; Tynes et al., 1985, 1986; Poulsen et al., 1986; Nagata et al., 1990; Hodgson & Levi, 1991; Venkatesh et al., 1992). Since those early studies, FMOs have been purified and/or cloned from multiple sources (Table 1). Until recently, it was thought that only 5 genes were expressed in mammals (Hines et al., 1994; Lawton et al., 1994; Cashman, 1995; Phillips et al., 1995). A sixth human gene, *FMO6*, identified by the Sanger Center chromosome 1 sequencing project, was demonstrated to be a psuedogene (Hines et al., 2002). These FMO genes are located in a cluster on the long arm of chromosome 1 (q23–25). The laboratories of Drs. Ian Phillips and Elizabeth Shephard have recently discovered a second FMO gene cluster, located also on chromosome 1. This second cluster in humans contains an additional 5 genes, all of which appear to be pseudogenes. Interestingly, in the mouse, this second gene cluster contains 3 genes which do not appear to be pseudo genes (Hernandez et al., 2004).

As with the CYP monooxygenase system, over the course of evolutionary plant–animal warfare, FMO developed broad substrate specificity at the expense of turnover. Both monooxygenases are capable of oxidizing thousands of plant alkaloids and other natural products as well as the thousands of synthetic therapeutic drugs. The CYP turnover number for most xenobiotics is 1–20 min^{-1} and the number is slightly higher for FMO, i.e., 30–60 min^{-1}. Although active toward many of the same substrates, CYP and FMO often produce distinct metabolites. It is recognized in the drug development field that potential concerns can arise if a drug contains a structural feature capable of being bio- activated by CYP to a toxic metabolite, e.g., an epoxide, oxon, or primary aryl *N*-hydroxy-amine. In general, FMO oxygenation results in metabolites with reduced pharmacological and toxicological properties.

As with every rule, there are exceptions. FMO can S-oxygenate thioureas to sulfenic acids which can undergo redox cycling or be further converted to the reactive sulfinic acid (Poulsen et al., 1979; Neal & Halpert, 1982; Ziegler, 1982; Krieter et al., 1984; Sabourin & Hodgson, 1984; Nagata et al., 1990; Decker & Doerge, 1991; Guo & Ziegler, 1991; Guo et al., 1992; Onderwater et al., 1999, 2004; Kim & Ziegler, 2000; Smith & Crespi, 2002; Henderson et al., 2004b).

The structure/function properties of the FMO family are still relatively unknown, as are the physiological function(s) of this enzyme (other than in xenobiotic metabolism), the impact of polymorphic expression, and the importance, relative to CYP, in the metabolism and efficacy of therapeutic drugs. In this review, we have attempted to summarize the current state of that knowledge and suggest possible avenues of further research.

2. Structure/function of flavin-containing monooxygenase

2.1. Catalytic mechanism and reactive oxygen species ROS generation

FMO belongs to a class of monooxygenases capable of generating a stable C4a peroxyflavin intermediate (Massey, 1994). This intermediate is stable for minutes to hours at 4 °C and can be observed spectrally (Beaty & Ballou, 1980; Ziegler et al., 1980; Williams et al., 1984; Jones & Ballou, 1986). In the first step of the catalytic cycle, FAD undergoes 2-electron reduction by NADPH (Fig. 1). The reduced flavin reacts rapidly with molecular oxygen to form the peroxyflavin and it is in this state, which the late Henry Kamin of Duke University likened to a “cocked gun,” that FMO may exist predominantly in the cell, waiting for a suitable nucleophile with which to react. This nucleophilic attack by the substrate on the FADOOH results in 1 atom of molecular oxygen being transferred to the substrate and 1 atom to form water. The rate-limiting steps in the catalytic cycle are thought to be the breakdown of the FADOH psuedobase or the release of $NADP^+$. In either case, it is important to note that, unlike the CYP monooxygenase system, substrate binding has no influence on V_{max}.

The FMO must have evolved a mechanism to protect nucleophilic sites (e.g., methionine, cysteine) from oxidative attack by the peroxyflavin. Furthermore, the structural features of the FAD pocket must be designed to minimize uncoupling/leakage of reactive oxygen species from the breakdown of FADOOH. As FMO is present at high concentrations in the endoplasmic reticulum of some tissues (up to 10% of the total microsomal protein in rabbit lung, Williams et al., 1985), a significant production of superoxide anion radical or hydrogen peroxide from the decomposition of the FADOOH would be detrimental. Rauckman et al. (1979) found that purified pig liver FMO (FMO1) produced superoxide anion radical at the rate of about 4% the total NADPH oxidized. Tynes et al. (1986) found hydrogen peroxide produced by the purified rabbit lung FMO (FMO2) at up to 41% of the total NADPH oxidized. The toxicological consequences of such ROS production by FMOs have not been investigated. The generation of hydrogen peroxide by FMO could also play an important physiological role in control of the overall redox state of the cell and in expression of genes controlled by hydrogen peroxide or the cellular redox potential. However, a caveat to this hypothesis of generation of ROS by FMO is that the purified enzyme may differ markedly from the native enzyme in the endoplasmic reticulum with respect to this “leakage” phenomenon. In the 1970s, Ziegler and Poulsen hypothesized that FMO may play a role in the synthesis of protein disulfide bonds through cysteamine oxidation (Poulsen & Ziegler, 1977; Ziegler et al., 1979). Cysteamine is one of a few endogenous substrates for FMO (see Section 2.4) and is oxidized to the disulfide cystamine. Interestingly, the yeast FMO has been shown to carry out such a cellular function, i.e., it functions in disulfide bond formation in protein synthesis and regulates thiol/disulfide ratios in the cell (Suh et al., 1999; Suh & Robertus, 2002). The yeast FMO gene 5′-promoter region contains an unfolded protein response element and transcription is regulated by this pathway (Suh & Robertus, 2000). In addition, yeast FMO enzyme activity is sensitive to the

redox state of the cell (Suh et al., 2000). The mutant yeast FMO exhibits enhanced sensitivity to reductive stress (Suh & Robertus, 2000).

2.2. Substrate specificity of enzyme-restricted substrate access for flavin-containing monooxygenase 2

The initial studies with the purified pig liverFMO(FMO1) established that this enzyme has a very promiscuous substrate specificity. Nitrogen-containing compounds, ranging from the size of trimethylamine to trifluoperazine- and sulfurcontaining compounds ranging from thiourea to sulindac sulfide, can be substrates. Any compound containing a soft nucleophile gaining access to the FADOOH can be a substrate. Compounds containing a single positive charge (e.g., tertiary amines) are excellent substrates. Many compounds containing a negative charge are excluded entirely or are poor substrates with exceptions such as sulindac sulfide and lipoic acid (Taylor & Ziegler, 1987; Hvattum et al., 1991; Attar et al., 2003). Zwitterions and compounds with more than 1 positive charge are typically not substrates, although some, like methionine, can be oxygenated, but the apparent K_m is above physiological levels (Park et al., 1992, 1994; Duescher et al., 1994; Elfarra, 1995; Krause et al., 1996, 2002, Ripp et al., 1997, 1999). Diamines, such as cadaverine and spermidine, are not oxygenated by FMO.

Upon purification and characterization of the first extrahepatic FMO, Tynes et al. (1985) and Williams et al. (1984) documented a number of important characteristics that distinguished rabbit lung FMO (FMO2) from the porcine liver enzyme. Among these differences was a markedly distinct substrate specificity. Previous studies employing rabbit lung microsomes had provided suggestive evidence for the existence of an FMO with distinct substrate specificity properties. Chlorpromazine and imipramine, both excellent substrates for porcine liver FMO, were not oxygenated by the rabbit lung enzyme (Ohmiya & Mehendale, 1984). The results with the purified rabbit lung FMO confirmed this lack of turnover with chlorpromazine and imipramine (Williams et al., 1984). Utilizing a series of 10-(*N*,*N*-dimethylaminoalkyl)- 2-(trifluoromethyl) phenothiazines with the alkyl side chain varying in length from 2 to 7 carbons, and a series of thioureas varying in size from thiourea to diphenylthiourea (Nagata et al., 1990), further characterized the differences between porcine liver FMO1 and rabbit lung FMO2. With the exception of the shortest side chain (C2), which had a K_m 4–5 times higher than the other derivatives, the length of the alkyl tertiary amine side chain made no difference in the kinetics of FMO1 N-oxygenation. In contrast, rabbit lung FMO2 exhibited no detectable activity until the side chain was at least 5 carbons in length. As the length of the side chain increased from 5 to 7 carbons, the K_m decreased such that it was equivalent to that of FMO1. With respect to the thioureas of different dimensions, again, the kinetics of porcine FMO1 S-oxygenation did not vary with substrate size. Rabbit FMO2 was incapable of S-oxygenation of 1,3-diphenylthiourea, but was active toward phenylthiourea and naphthylthiourea. Based on these results, Nagata et al. (1990) estimated that the rabbit lung enzyme had a restricted substrate access channel with the FADOOH active site 6–8 Å below the surface of a channel that was no wider than 8 Å. The lack of activity of rabbit FMO2 toward imipramine and chlorpromazine are consistent with this model. Thioureas of various sizes have been used to demonstrate the existence of multiple FMO enzymes in various tissues (Nagata et al., 1990; Guo et al., 1992; Shehin-Johnson et al., 1995; Kim & Ziegler, 2000). The activity of rabbit FMO2 toward alkyl 2-naphthyl sulfides of increasing chain length confirm this restricted substrate access channel in FMO2 (Fisher & Rettie, 1997).

In collaboration with Dr. John Cashman (Human Bio-molecular Research Institute, San Diego, CA), we have recently documented that the size restriction on substrates also applies to human FMO2. No activity is observed with the phenothiazine derivative with a 3 carbon alkyl side chain, but the K_m for the 5 and 8 carbon derivatives were 18 and 10 μM, respectively (Fig. 2). In a similar manner, as with rabbit FMO2, the human enzyme is active toward thioureas with

small substituents, but is incapable of S-oxygenating 1,3- diphenylthiourea (Henderson et al., 2004b). However, one must be cautious in extrapolating substrate specificity of a particular FMO across species. Kim and Ziegler (2000) demonstrated that the human FMO1 is not as promiscuous as the pig FMO1 and cannot S-oxygenate 1,3-diphenylthiourea. The FADOOH access channel is probably not as deep as FMO2, as soft nucleophiles extending 4.2–5 A° from a bulky ring system (imipramine, orphenadrine, and chlorpromazine) can be oxygenated by human FMO1, but not FMO2 (Kim & Ziegler, 2000). A second precaution with respect to predicting FMO enzyme substrate specificity is that factors other than size and charge must play a role, but these parameters are not well understood. An example is the high selectivity observed with human FMO3, compared to the other FMO enzymes, in the N-oxygenation of the important constitutive substrate trimethylamine (Lang et al., 1998). We also do not know what structure/function factors result in the observed restricted substrate specificity for FMO5 (Rettie et al., 1994;Overby et al., 1995;Cherrington et al., 1998). FMO5 exhibits little activity toward methimazole or other typical FMO substrates, but does carry out the N-oxygenation of short-chain aliphatic primary amines such as *N*-octylamine. The substrate specificity for FMO4 also appears somewhat restricted, but even less is known about this FMO, probably due in part to difficulties in heterologous expression ofFMO4 (Itagaki et al., 1996). FMO4 exhibits a higher molecular weight than other FMO enzymes, but in some tissues, a smaller protein is present, apparently due to alternative splicing (Lattard et al., 2003a,2003b). Because of their limited substrate specificity (FMO4 and FMO5) and relatively low expression levels in most tissues (FMO4), these enzymes are currently not thought to play an important role in drug metabolism.

A second striking difference with respect to substrate specificity between purified porcine liver FMO1 and rabbit lung FMO2 is observed with primary aliphatic amines. These amines, typified by *N*-octylamine are not substrates of the porcine liver FMO1, but serve as positive effectors of catalytic activity (Williams et al., 1984, Tynes et al., 1985, 1986; Poulsen et al., 1986). In contrast, primary alkylamines are readily oxygenated by rabbit FMO2, with the K_m decreasing with increasing chain length between C8 and C12. The initial oxygenation results in production of the *N*-hydroxy primary amine, which is a much better substrate than the parent amine (e.g., the K_m for *N*-hydroxy-dodecylamine is 2 orders of magnitude lower than dodecylamine). The product from this second oxygenation is the oxime which is formed with high stereoselectivity toward production of the *cis* isomer (Poulsen et al., 1986).

Other notable structure/function distinctions between FMO1 (and to some degree FMO3) and FMO2 can be seen in properties such as thermolability, activation by divalent cations, and inhibition by anionic detergents (Devereux & Fouts, 1974; Devereux et al., 1977; Sabourin & Hodgson, 1984; Kaderlik et al., 1991; Lawton et al., 1991; Venkatesh et al., 1991a, 1992; Williams, 1991; Krueger et al., 2001, 2002a). FMO1 and FMO3 are readily inactivated by incubation at temperatures of 45–50 °C for as few as 5 min and are inhibited at low concentrations of anionic detergents such as sodium cholate or fatty acids. FMO2, on the other hand, is relatively stable at 45–50 °C, especially in the presence of NADPH, and concentrations of sodium cholate as high as 1% do not inhibit catalytic activity. It should be noted that these are general observations on a limited number of enzymes and, again, one must be cautious in extrapolation across species.

In summary, some generalities concerning individual FMO substrate specificity are observed. FMO4 and FMO5 have a very restricted substrate specificity and probably do not contribute significantly to drug metabolism in humans. FMO1 has the broadest substrate specificity of any of the FMOs and FMO2 appears to be the most restricted (based on size); FMO3 is intermediate. An absolute structural requirement for an FMO substrate is a soft-nucleophilic heteroatom, typically a nitrogen or sulfur, with fewer examples of selenium- or phosphorus-containing drugs also known. Uncharged compounds or compounds with a single positive

charge are the best substrates. Compounds with a single negative charge can be substrates, but the charge must be located a certain distance from the oxygenation site. Compounds with more than a single charge are almost universally excluded from the FADOOH site. Although some predictions, based on structure, can be made concerning the potential for a particular drug to be oxygenated by FMO, this question still has to be determined empirically.

2.3. Poulsen and Ziegler model predictions for protein structure and functional domains

To date, the tertiary structure of FMO has not been solved; however, Ziegler and Poulsen (1998) proposed a model based on comparison with the solved structures of other flavoenzymes. Using the families of structurally similar proteins (FSSP) database (Holm & Sander, 1994), FMOs are members of the flavocytochrome *c* sulfide dehydrogenase (41.7.1.1.1.1) subfamily of flavoenzymes, which includes a number of NAD(P)H-dependent oxidases and reductases for which the tertiary structure has been solved. The members of this family catalyze either 2 electron reduction of dioxygen or disulfides. Although the primary structure of family members is not highly conserved, tertiary structures elucidated to date are similar and all members are dimers composed of identical subunits.

Since structural similarities abound among family members, Ziegler and Poulsen (1998) applied the known structure of family member *Escherichia coli* glutathione reductase (GR; Thieme et al., 1981) to pig FMO1 and subsequently human FMO3 (Ziegler, 2002). An alignment of primary structures was built around regions of high conservation, which serve as anchor points in the structure. The function known for a given segment of GR was assigned the same function in FMO for the aligned region. The PRINTS database (Attwood et al., 2003) catalogs protein fingerprints, which are conserved motifs that characterize a protein family. This database currently recognizes 13 motifs characterizing FMOs (FMO PRINTS signature [FMOXYGENASE] motifs; Table 2), rather than the 7 originally documented by Ziegler and Poulsen (1998). Using the amino acid sequence for rabbit FMO2 (Accession M32029; Lawton et al., 1990) as the model and the current PRINTS database release, other common elements include FAD-dependent pyridine nucleotide reductase (FADPNR), pyridine nucleotide disulfide reductase class-I (PNDRDTASEI) and adrenodoxin reductase (ADXRDTASE) signature homologies. GR is classified with both the FADPNR and PDRDTASEI signature groups. GR and all of the members in these groups have an N-terminal FAD-binding domain followed by an NAD(P)H-binding domain, a central domain and a C-terminal interface domain.

The basic elements of dinucleotide-binding motifs (DBMs) have been known for many years. The classic Rossmann (Rossmann et al., 1974; βαβ) fold of DBMs has been described in detail (Wierenga et al., 1985) and contains the GXGXXG and GXGXX(G/A) motifs which bind the ADP moiety of FAD and NAD(P)H, respectively (FMOXYGENASE motifs 1 and 9), in addition to other conserved residues. Other, more recently recognized, motifs common to flavoproteins with 2 DBMs (Vallon, 2000) include the GG motif which stabilizes FAD binding (FMOXYGENASE motif 2), 2 ATG motifs (FMOXGENASE motifs 7 and 11) that form the linkage between the FAD and NADPH domains, respectively, and the linkage between the NADPH and active site domains. The final common element identified by Vallon is the GD motif (FMOXYGENASE motif 12) that binds the FAD flavin moiety; it should be noted, however, that with this motif, conservation is limited and requires experimental evidence to confirm FAD binding by this region. All of these motifs are shown with the PRINTS database information cataloged in Table 2. The ATG motifs are composed of an acid residue, 4 to 6 variable amino acids and the ATG; only the G shows complete conservation among FMO relatives. The FATGY motif recognized for years as a conserved component of nearly all mammalian FMOs (FMO4 proteins use FTTGY) has at its core this second ATG motif. The ATG fingerprint, $D(X)_4ATG$, retains only the D and G residues in many of its flavoenzyme

relatives. However, N-hydroxylating siderphore enzymes from bacteria and fungi have retained the closest reiteration of the FATGY motif (Stehr et al., 1998) which can be written as $D(X)_3(L/F)ATGY(X)_4P$, where L can be substituted for F among siderphore enzymes. Like other FMO relatives these enzymes also bind FAD (via a modified GXGXXP motif) and NADPH.

Another fingerprint has been identified among FMOs upon comparison of sequences with those from Baeyer–Villiger monooxygenases (BVMOs; Fraaije et al., 2002), bacterial or fungal flavoenzymes that use dioxygen and NAD(P)H as an electron donor to convert a ketone to an ester. The fingerprint thus identified among mammalian FMOs is indicated above the expanded version of FMOOXYGENASE motif 8 (Table 2), as FXGXXXHXXXY. This fingerprint occurs just before the NAD(P)H-binding GXGXX(G/A) motif. BVMOs have a slightly modified motif that substitutes W for Y. When Fraaije et al. (2002) used site-directed mutagenesis of 4-hydroxyacetophenone monooxygenase, a BMVO enzyme, to convert the central H→A or the terminal W→Y of this motif, they found that the W→Y protein was both insoluble and inactive. The H→A protein was a soluble dimer, but prone to losing some of the FAD cofactor during purification; furthermore, the mutant had almost no activity. Cheesman et al. (2003) mutated the homologous H of cyclohexanone monooxygenase, another BVMO, to Q. These authors found only limited evidence of impaired FAD binding with the H→Q mutant, but did find that enzyme activity was only 10% that of the wild-type enzyme. Kinetic studies indicated an 11- fold decrease in enzyme efficiency (V_{max}/K_m) that was only partially explainable as a decreased binding affinity for NADPH. Thus, FMOXYGENASE motif 8, contributes to NADPH binding and appears to have an additional function as well.

There are a number of studies that have sought to understand why particular mutations made to FMO proteins alter the function of the protein, and some of these studies verify aspects of the proposed structural model. Obvious examples of this are experiments that demonstrate that site-directed mutagenesis of the G residues of the conserved GXGXXG from the putative DBM for FAD, does, in fact, cause the loss of FAD binding and concomitant loss of enzyme activity (Lawton & Philpot, 1993; Kubo et al., 1997). More recently, Zhang et al. (2003) determined that an individual presenting with trimethylaminuria (TMAU) had 2 mutations, one of which encoded p.E32K. The E residue corresponds to the conserved (E/D) that is part of the GG motif (Table 2, FMOXYGENASE motif 2) that stabilizes FAD binding. Although Zhang et al. (2003) did not confirm that expressed p.E32K had suboptimal FAD content, they were unable to detect either N- or S-oxidation with FMO3 substrates, which would be the expected outcome in the absence of FAD binding. There are 2 further causative mutations of TMAU that occur just downstream of the GXGXX(G/A) motif, but within the βαβ-fold for NAD(P)H, p.I199T (Zschocke et al., 1999) and p.R205C (Fujieda et al., 2003). Both of these mutations are at positions described by Wierenga et al. (1985) as being tolerant of any amino acid. While the relevant determinations of NAD(P)H binding and K_m were not given for the TMAU mutations, due to the location of the mutations, it is likely that these alterations are interfering with NADPH binding, although the possibility of a second FMO-specific function occurring in this motif cannot be eliminated at present. A final example of a mutation that verifies part of the proposed structural model is the p.M434I single-nucleotide polymorphism (SNP) that also causes TMAU (Dolphin et al., 2000). N- and S-oxygenations performed by this protein are drastically reduced, despite the fact that FMO1 enzymes utilize an I residue at this location and FMO2 enzymes utilize L at the same position. The p.M434I residue is expected to lie in or near to the FMO enzyme specific substrate cleft (Table 2), so it is not surprising that a residue tolerated by FMO1 would alter the activity of FMO3.

The proposed structural model may be useful in preliminary predictions of the general 3-dimensional structure of FMO, assignment of function to specific regions of the sequence and generation of hypotheses that can be tested on expressed mutant proteins; however, the model

also has serious limitations. For example, while we can with fair precision assign a function such as stabilization of FAD binding to FMOXYGENASE motif 2, that cannot be interpreted to mean that no other function exists for that sequence. The comparable motif of GR, in addition to FAD binding stabilization, also contains 2 C residues involved in the redox-active disulfide bond of this protein, but disulfide bonds do not exist for mammalian FMO. Dual-function fingerprints, and interactions between residues that are distant in the sequence alignment but interact in the tertiary structure, will abound. Because structural features that are unique to FMO, and yet define FMO as a family, are least amenable to modeling a priori, crystallization of an FMO remains essential to gaining an in-depth understanding of the structure and function of this family. The only bacterial flavin monooxygenase whose crystal structure has been solved is phenylacetone monooxygenase (PAMO; Malito et al., 2004). The authors found significant homology with human GR (ca. 20% identity). PAMO may provide important insights into mammalian FMO structure/function. Again, only the acquisition of a structure derived from crystals of a mammalian FMO will settle the issue definitively.

2.4. Physiological function(s) of flavin-containing monooxygenase: endogenous substrates and association with disease (Table 3)

As discussed previously, the charge restrictions for access to the substrate channel leading to FADOOH exclude many potential nucleophilic endogenous substrates from FMO-dependent oxygenation. There are a number of notable exceptions, however. For the purpose of the present discussion, “endogenous substrate” will refer to a chemical synthesized in the body or as a result of conjugation of a xenobiotic with an endogenous nucleophile such as cysteine. Dietary constituents, such as alkaloids, allyl sulfides, amines, etc., are not considered to be “endogenous” substrates. In some cases, this distinction is blurred and admittedly somewhat arbitrary. For example, we will consider the biogenic amine phenethylamine as an endogenous substrate, although a significant amount of human exposure can also be dietary.

2.4.1. Nitrogen-containing endogenous substrates—The biogenic amines, phenethylamine and tyramine, are N-oxygenated by FMO to produce the *N*-hydroxy metabolite, followed by a rapid second oxygenation to produce the *trans*-oximes (Lin & Cashman, 1997a, 1997b). This stereoselective N-oxygenation to the *trans*-oxime is also seen in the FMO-dependent N-oxygenation of amphetamine (Cashman et al., 1999), but differs from that seen with 10-*N*-(*n*-octylamino)- 2-(trifluoromethyl) phenothiazine (Lin et al., 1996) and of straight-chain aliphatic amines discussed previously (Poulsen et al., 1986), for which the product is the *cis*-oxime. Interestingly, FMO2, which very efficiently N-oxygenates *N*-dodecylamine, is a poor catalyst of phenethylamine N-oxygenation. The most efficient human FMO in phenethylamine N-oxygenation is FMO3, the major FMO present in adult human liver; the K_m is between 90 and 200 μM (Lin & Cashman, 1997b). The oximes appear to have little pharmacological activity, and therefore FMO-mediated N-oxygenation of biogenic amines appears to be a mechanism for inactivation. Brain contains active FMO (Bhamre et al., 1993, 1995; Bhagwat et al., 1996a, 1996b; Blake et al., 1996; Kawaji et al., 1997) and conversion of biogenic amines in the brain by FMO (Lin & Cashman, 1997a) could be of clinical significance.

Trimethylamine is another example of an endogenous compound that is also present in the diet (primarily as trimethylamine N-oxide from fish, which is reduced by gut bacteria to the parent amine). Trimethylamine is primarily formed in vivo from the breakdown of choline and is N-oxygenated by FMO (Gut & Conney, 1993; Lang et al., 1998; Lambert et al., 2001; Cashman et al., 2004). The product, as with all FMO-mediated oxygenations of tertiary amines, is the N-oxide. Trimethylamine is extremely odorous, whereas, the N-oxide has little or no detectable odor. Failure to efficiently N-oxygenate trimethylamine leads to the genetic disease known as trimethylaminuria (see Section 3.1).

2.4.2. Sulfur-containing endogenous substrates—Sulfur-containing endogenous substrates for FMO have also been documented (Poulsen, 1981). Sulfhydryls, such as cysteamine, are S-oxygenated to the disulfide (cystamine). Cysteamine has been used therapeutically as a radioprotector (Biaglow et al., 1984) and is the only approved treatment for pediatric cystinosis (Belldina et al., 2003). Cysteamine also can regulate various hormones; it is a potent inhibitor of somatostatin and has been shown to affect circulating growth hormone levels in pigs (McElwain et al., 1999). The physiological role of cysteamine S-oxygenation by FMO is not known. A number of years ago, Ziegler and Poulsen hypothesized a role for FMO in protein disulfide bond formation through oxidation of cysteamine (Ziegler et al., 1979). FMO oxygenation of cysteamine may serve to help control the overall thiol/disulfide redox state of the cell which, in turn, controls many important pathways. However, this is entirely speculation at this point. Another function may simply be a protective one. Cysteamine, at concentrations as low as 39 μM, is toxic to cells, apparently through the transition metal-dependent formation of hydrogen peroxide (Jeitner & Lawrence, 2001). It has been postulated that cysteamine can reach and exceed these concentrations (perhaps as high as mM concentrations) through transport into the cell as a mixed disulfide with cysteine (required to maintain high intracellular glutathione levels), followed by reduction intracellularly. FMO oxygenation of cysteamine to cystamine and transport out of the cell may represent a detoxication mechanism. By this indirect mechanism, FMO may control H_2O_2 levels in the cell and the expression of genes regulated by H_2O_2, sulfhydryl/disulfide ratios and general redox state (Khomenko et al., 2004).

Lipoic acid is another example of an “endogenous” substrate also available through the diet as a dietary antioxidant supplement (Bustamante et al., 1998) and as a therapeutic agent (Smith et al., 2004). Lipoic acid is a cofactor for α-ketoglutarate and pyruvate dehydrogenases. Recent evidence suggests that lipoic acid can play an important role in protection of mitochondria during aging or even in reversal of age-associated declines in mitochondrial function (Hagen et al., 2002). FMO catalyzes the S-oxygenation of the disulfide lipoic acid, as well as lipoamide. Recently, we have found that human FMO2 exhibits activity toward lipoic acid with a K_m of 71 μM (Henderson et al., unpublished). The major urinary metabolites of lipoic acid in mice, rats, dogs and humans appear to be derived from mitochondrial β-oxidation or reduction to lipoamide followed by methylation and sulfoxidation (Schupke et al., 2001). The potential role of FMO in the sulfoxidation of the methyl sulfides of lipoic acid is not known at this time.

As discussed previously, FMO-mediated oxygenation of a number of potential endogenous compounds are prohibited due to charge restrictions in the FADOOH-substrate access channel. However, FMO3 will oxygenate methionine, but the K_m is at mM concentrations which are probably not physiologically relevant. Many xenobiotics are metabolized by glutathione *S*-transferases and these compounds are then excreted as the cysteine S-conjugates or mercapturic acids. A number of cysteine S-conjugates of xenobiotics are S-oxygenated by FMO (Table 3), but again the K_m values are in the low mM range which may call into question the importance of such metabolism in vivo. The Se of selenocysteine conjugates is also oxygenated by FMO (Rooseboom et al., 2001). Interestingly, the sulfoxidation of farnesyl cysteine is catalyzed by FMO with a K_m in the low μM range (Park et al., 1994).

2.4.3. Association of flavin-containing monooxygenase with human disease—As discussed in Section 3.1 on genetic variants, currently the association of FMO expression and human disease is only established for the genetically inherited disease known as trimethylaminuria. Numerous studies have correlated altered susceptibility to a number of diseases, the existence of particular alleles and the expression of certain phase 1 and/or phase 2 drug metabolizing enzymes (e.g., Rosvold et al., 1995; Taioli et al., 1998; Ford et al., 2000; Wu et al., 2001). In the case of CYP, the wide interindividual variation in expression is due to both genetic and environmental factors. As FMO is not markedly regulated by such

environmental factors, one could predict interindividual variability in FMO enzyme expression would be predominantly genetic in origin and it may be possible to more clearly delineate correlations (or lack thereof) between expression of wild-type FMO or particular FMO allelic variants and susceptibility to disease. Hepatic total FMO activity is enhanced in mouse models of type I and type II diabetes (Rouer et al., 1987, 1988). Conversely, expression of FMO5 is markedly down-regulated in the liver of humans with type II diabetes (Takamura et al., 2004). Alterations in FMO expression and activity in diabetic patients could be of significance in drug therapy (Section 4). A global gene expression approach with patients diagnosed with atrial fibrillation documented a significant increase in the expression of FMO1 (Kim et al., 2003). There has also been suggestive evidence presented that FMO may be associated with sideroblastic anemia (Barber et al., 2000). Finally, expression of certain FMO enzymes in some neoplastic tissues has been observed (Rebhan et al., 1997). Our knowledge of the physiological function of FMOs and the relationship of FMO deficiency or over expression to disease susceptibility is still in its infancy.

3. Genetic variants and polymorphisms

3.1. Genetic variants in flavin-containing monooxygenase 3 and trimethylaminuria

Trimethylaminuria (TMAU), previously referred to as “Fish-Odor Syndrome”, is manifested clinically by a body odor resembling rotten fish and is accompanied by various psychosocial abnormalities (Ayesh & Smith, 1990; Treacy et al., 1998; Mitchell & Smith, 2001). This disease is classified by NIH as a rare disease and has been the focus of 2 international meetings sponsored by the NIH Office of Rare Diseases. The actual incidence of TMAU, however, may be underreported. Due to the paucity of information available to physicians, this disease is rarely diagnosed in a timely fashion and the symptoms for patients are often attributed to poor hygiene.

This disease has probably been around for centuries. In the Mahabharata, in an epic tale perhaps dating back to as early as 1400 BC, Satyavata is described as a young woman ostracized from society because she smelled like rotting fish (Mitchell & Smith, 2001). Shakespeare likely was referring to a TMAU patient in a scene from The Tempest (II. ii. 26–29). As the name implies, the condition is caused by an excess of the extremely odorous trimethylamine in the urine, sweat, and breath of patients. Trimethylamine is formed endogenously from the breakdown of choline and the reduction of trimethylamine N-oxide present in high concentrations in diets rich in fish. Trimethylamine is normally metabolized to the N-oxide, a metabolite with no detectable offensive odor, by FMO in the liver (Sardas et al., 1996; Mitchell et al., 1997). Studies in vitro with expressed FMO enzymes have shown FMO3 to be selective in the N-oxygenation of trimethylamine (Lang et al., 1998). A number of allelic variants have been associated with the incidence and severity of TMAU in patients (Zhang et al., 1996, 2003; Cashman et al., 1997, 2000, 2001, 2003; Dolphin et al., 1997; Kang et al., 2000; Murphy et al., 2000; Forrest et al., 2001; Cashman, 2002; Cashman & Zhang, 2002; Park et al., 2002; Hernandez et al., 2003; Lattard et al., 2003a, 2003b). These individuals can be diagnosed by urinary ratios of trimethylamine N-oxide to trimethylamine following a challenge dose of choline. Most individuals excrete 95% or more of the trimethylamine as the N-oxide. The severity of the disease can be predicted to be greater with a lower ratio, which appears to be related to the type of FMO3 gene mutation. Interestingly, the incidence of TMAU appears to be higher in females (although there could be a bias at diagnosis) and patients often report the symptoms to be worse during menstruation. Once diagnosed, the most effective treatments appear to be dietary restriction (low choline, fish) and/or antibiotic treatment (inhibits bacterial reduction of trimethylamine N-oxide in the intestine). Recently, treatment with charcoal or the deodorant copper chlorophyllin has been demonstrated to be effective in some patients (Yamazaki et al., 2004). Interestingly, reports of transient TMAU in young children have been reported. This phenomenon may be explained by the observation that the FMO with high

activity toward trimethylamine, FMO3, is not expressed in fetal liver, but is induced after birth (0–8 years of age; Koukouritaki et al., 2002).

Of particular significance for this review is that individuals homozygous for certain FMO3 allelic variants (e.g., null variants) also demonstrate impaired metabolism toward other FMO substrates including ranitidine, nicotine, thio-benzamide, and phenothiazine derivatives (Table 4; Cashman et al., 1995,2000;Kang et al., 2000;Cashman, 2002;Park et al., 2002;Lattard et al., 2003a,2003b). The rare null variants are present in patients with TMAU, whereas variants resulting in a less efficient enzyme (e.g., E158K, E308G) may not exhibit a phenotype. In at least one study, in vivo phenotyping of impaired drug metabolism using ranitidine as the substrate correlated to genotyping for such FMO3 allelic variants (Kang et al., 2000). These results support an important role for FMO in human drug metabolism and highlight the need for further studies on pharmacogenetics of FMOs and drug metabolism. Other FMO3 allelic variants which produce inactive FMO3 (Table 4) include P153L (Dolphin et al., 1997;Treacy et al., 1998); E305X (Treacy et al., 1998); M66I (Treacy et al., 1998;Akerman et al., 1999a); R492W (Akerman et al., 1999a); A52T, R387L, E314X (Akerman et al., 1999b); I199T (Zschocke et al., 1999); V143E (Basarab et al., 1999); N61S, M434I (Dolphin et al., 2000); V58I (Kubota et al., 2002); D198I and R205C (Fujieda et al., 2003).

Inherited genetic defects in hepatic FMO metabolism of trimethylamine have also been documented in livestock. A serious problem developed in the UK with certain breeds of chickens, notably Brown Leghorn and Rhode Island Red, fed diets containing high levels of choline. These chickens laid a high percentage of eggs with a “fishy taint” that were unmarketable (Butler & Fenwick, 1984). This malodor was attributed to high levels of trimethylamine deposition in the eggs. Fortunately, the problem has largely been solved by diet modification and breeding programs. The second example is with some dairy cows that produce milk with a definitive off-flavor and smell, again attributed to high levels of trimethylamine deposition (Lunden et al., 2002). It should be noted here that humans may be particularly susceptible because, unlike most other animals, FMO3 is the major hepatic FMO in the adult.

3.2. Genetic polymorphisms of flavin-containing monooxygenase 2

FMO2 is the major lung FMOexpressed in most mammals including non-human primates (Yueh et al., 1997; Dolphin et al., 1998; Krueger et al., 2001). As discussed in Section 2.2, FMO2 exhibits a number of distinct properties of substrate specificity, thermostability, resistance to detergent inhibition, etc. Our initial attempts to characterize FMO2 protein expression in human lung were unsuccessful. Subsequently, Dolphin et al. (1998) published the sequence of human FMO2 and documented a very interesting genetic polymorphism in expression. All of the Caucasians and Asians genotyped to date have 2 alleles with a c. 1414C→T transition replacing a Gln (CAG, *FMO2*1*, p.Q472) with a premature stop codon (TAG, *FMO2*2A*, p.X472; Dolphin et al., 1998; Whestine et al., 2000; Krueger et al., 2002b; Furnes et al., 2003). This truncated protein is not found in human lung samples; expressed p.X472 does not incorporate FAD, is catalytically inactive, and is probably rapidly destroyed due to incorrect folding (Whestine et al., 2000; Krueger et al., 2002a). Interestingly, 27% of individuals of African descent (Dolphin et al., 1998; Whestine et al., 2000) and 5% of Hispanics (Krueger et al., 2004) possess at least 1 allele coding for the full length, enzymatically active protein (FMO2.1, p.Q472). Individuals genotyped as possessing at least 1 *FMO2*1* allele express full length, catalytically active protein which can be detected by immunoquantitation. Furnes et al. (2003) have documented additional variants in FMO2 (as well as FMO1, FMO4, and FMO5) in African–Americans. We have recently evaluated the occurrence of 4 common FMO2 variants (c.211_213dup- GAC, c.337delG, c.584C→T, and c.1239T→G) identified in African–Americans by Furnes et al. (2003) in Hispanics and

expressed each to determine the probable impact on drug metabolism (Krueger et al., in press). Only the expressed p.N413K (c.1239T→G) variant had full catalytic activity (Table 4). The allelic frequency of these variants was lower in the Hispanic population we studied, compared to the African–American population in the Furnes et al. (2003) study, although this latter study examined fewer individuals (50). Furthermore, we have recently demonstrated through haplotype determination and inferred haplotype determination that these variants are located on the major c.1414T allele and thus would not significantly impact the activity of the full-length functional enzyme produced by the c.1414C allele (Krueger et al., in press). Interestingly, the FMO2 gene is also truncated in laboratory, but not in wild, strains of rats (Lattard et al., 2002), and the use of *R. norvegicus* as a model for the human FMO2 genetic polymorphism has been proposed (Hugonnard et al., 2004).

Our laboratory is now addressing the hypothesis that individuals with the *FMO2*1* allele metabolize drugs and xenobiotics differently than individuals with no functional FMO2 in the lung. FMOs bioactivate thioureas through S-oxygenation to toxic sulfenic and/or sulfinic acid metabolites (Poulsen et al., 1979; Neal & Halpert, 1982; Ziegler, 1982; Sabourin & Hodgson, 1984; Nagata et al., 1990; Decker & Doerge, 1991; Decker et al., 1992; Guo et al., 1992; Kim & Ziegler, 2000; Smith & Crespi, 2002). The sulfenic acid is capable of undergoing redox cycling following conjugation with glutathione, resulting in oxidative stress due to depletion of reduced glutathione and NADPH (Hardwick et al., 1991; Decker & Doerge, 1992; Onderwater et al., 1998, 1999, 2004; Smith & Crespi, 2002; Henderson et al., 2004b). This is thought to be an important mechanism of thiourea toxicity in lung as well as in liver (Boyd & Neal, 1976; Lee et al., 1980; Scott et al., 1990; Hardwick et al., 1991; Ziegler-Skylakakis et al., 1998). Human FMO2 is very active toward bioactivation of small molecular weight thioureas (Henderson et al., 2004b) and one would predict that individuals expressing the *FMO2*1* allele would be at enhanced risk of toxicity following exposure.

In the case of thioether-containing organophosphate pesticides, the presence of the *FMO2*1* allele would be predicted to have the opposite (protective) effect. Organophosphates are bioactivated by desulfuration of the phosphorothionate sulfur to produce the oxon, a metabolite that is orders of magnitude more potent in cholinesterase inhibition (Neal & Halpert, 1982; Jokanovic, 2001). FMO-dependent oxidation of the thioether moiety produces the sulfoxide, a pathway that represents detoxication compared to oxon production (Hajjar & Hodgson, 1980, 1982; Kulkarni & Hodgson, 1984; Osimitz & Kulkarni, 1985; Smyser et al., 1985; Tynes & Hodgson, 1985b; Kinsler et al., 1988, 1990; Levi & Hodgson, 1988; Hodgson & Levi, 1992; Buronfosse et al., 1995; Hodgson et al., 1998; Usmani et al., 2004). Again, we have found human FMO2 to be very effective at S-oxygenation of the thioether of phorate and disulfoton (Henderson et al., 2004a), as has been previously shown for other FMO enzymes and for the mouse FMO2 (Karoly & Rose, 2001). Thus, in individuals expressing full-length, functional FMO2, we hypothesize that the metabolite profile should be shifted in favor of formation of the sulfoxide, rather than the more toxic oxon, resulting in reduced toxicity.

3.3. Genetic variants and polymorphisms in flavin-containing monooxygenase 1

In humans, FMO1 is the major FMO in fetal liver and adult kidney and intestine. The human is a very interesting species with respect to developmental regulation of FMO1 and FMO3. FMO1, the major FMO in liver of most adult mammals, is expressed at relatively high concentrations in human fetal liver, but shortly after birth, expression is reduced to almost zero; the signal for termination of expression is related to the parturition and not to gestational age (Hines & McCarver, 2002; Hines et al., 2003). FMO1 may play an important role in extrahepatic drug metabolism in humans as well as in the liver of the fetus exposed to numerous potential xenobiotic substrates in utero. Currently, there are ~20 allelic variants of human FMO1 described by Furnes et al. (2003) and Hines et al. (2003) and the GeneSNPs database.

The enzymatic activity of some of the FMO1 variants are given in Table 4. Based on the work of Hines et al. (2003), some of these allelic variants are probably rare alleles (H97Q, I303V, I303T, R502X) and may not contribute significantly to interindividual differences in FMO1 expression. On the other hand, a much more common variant (*FMO1*6*) caused by a C→A transversion in the upstream promoter region (−9,536), may impact FMO1 expression as it renders a Yin Yang basal promoter incapable of binding YY1 (Hines et al., 2003). The allelic frequency of *FMO1*6* is 13, 11, and 30% in African–Americans, northern European–Americans, and Hispanic–Americans, respectively (Hines et al., 2003).

4. Role of flavin-containing monooxygenase in drug metabolism

FMO and CYP share a number of similarities with respect to tissue, cellular, and organelle expression, the utilization of oxygen and NADPH as cofactors, and activity toward many of the same drugs. In order to determine in vitro the relative contribution of FMO versus CYP in the oxygenation of a particular drug, a number of approaches have been used. The activity of any and all microsomal CYPs can be inhibited by titration with antibody to NADPH CYP reductase, carbon monoxide, or addition of detergent. There are also CYP-selective antibodies and chemical inhibitors to dissect out the contribution of individual CYPs. Unfortunately, antibodies directed toward FMOs typically inhibit catalytic activity weakly, if at all, and the only chemical inhibitors are competitive substrates. The most commonly used alternative substrate to inhibit FMO in vitro (Mani et al., 1993; Narimatsu et al., 1999; Rawden et al., 2000; Pike et al., 2001; Attar et al., 2003; Wynalda et al., 2003; Virkel et al., 2004) and in vivo (Ruse & Waring, 1991; Nace et al., 1997; Wang et al., 2000) has been methimazole (see next section for further discussion of methimazole). FMO activity is unaffected by non-ionic detergent or carbon monoxide, but most FMOs are readily inhibited by short incubations at temperatures of 45–50 °C. In order to estimate the relative contribution of FMO versus CYP, the standard approach is to utilize a combination of these approaches, e.g., preincubation at elevated temperature and incubation with and without a competing FMO substrate. If the results are consistent, one can get a fairly accurate estimate of the relative contributions of these 2 monooxygenases. A more direct approach is to perform incubations of the test drug with individual expressed CYP and FMO enzymes now available commercially. By this approach, one can determine catalytic efficacy for individual FMOs. Another useful approach is to utilize banks of human liver microsomes with known levels of each CYP and FMO and correlate metabolism with the level of the individual enzyme. Finally, the relative importance of FMO and CYP in oxidative metabolism of a particular drug will depend not only upon the catalytic efficiency of that particular enzyme but also upon the relative abundance in the organ being studied. For example, if FMO3 and CYP2D6 had similar kinetics toward a drug, based on the relative abundance in liver, one would predict a greater contribution from FMO3 (perhaps even first pass hepatic metabolism).

It is somewhat more difficult to get an accurate assessment of the relative contribution of FMO in metabolism of a given drug in vivo (Damani & Nnane, 1996). Measurements of FMO-specific metabolites of nicotine (Berkman et al., 1995) and caffeine (Chung & Cha, 1997; Park et al., 1999; Chung et al., 2000) have both been proposed as possible approaches to phenotyping individuals. In the case of nicotine, the N-oxide represents a minor metabolite in urine and the fact that tertiary amine N-oxides are readily reduced in vivo complicates the interpretation of such assays with respect to phenotyping of individuals. Caffeine is very attractive as a potential substrate for phenotyping as one could theoretically simultaneously phenotype an individual for FMO, CYP1A2, xanthine oxidase, and *N*-acetyltransferase. However, subsequent examination has raised serious doubts about the utility of caffeine for FMO phenotyping (Lang & Rettie, 2000; Rettie & Lang, 2000). Ranitidine metabolism correlates with FMO3 phenotype (Park et al., 2002) and may provide promise in phenotyping individuals for metabolism of other FMO3 substrates.

In animal models, pretreatment with methimazole to inhibit FMO-mediated drug metabolism has been the most common approach (Ruse & Waring, 1991; Nace et al., 1997; Wang et al., 2000). Methimazole is not specific for inhibition of FMO and, in fact, is used clinically as an anti-thyroid drug as it inhibits thyroid peroxidase (Engler et al., 1982). In addition, methimazole can exhibit toxicological properties itself that may confound interpretation of the data (Brittebo, 1995). With respect to distinguishing FMO-from CYP-mediated metabolism, results with methimazole should be interpreted with caution as methimazole can be a substrate for CYPs (Kedderis & Rickert, 1985) and FMO-mediated S-oxygenation of methimazole produces the reactive sulfenic acid which inhibits CYP activity (Kedderis & Rickert, 1985). Thiourea has also been used in vitro to inhibit FMO-mediated activity, but again, due to the reactivity of the sulfenic acid of thiourea, interpretation of such results is not straightforward.

The relative contribution of individual FMO enzymes in the metabolism of a given drug is difficult to determine as, to date, there are few if any FMO family-specific or even selective substrates. Thus, one cannot phenotype an individual for a given FMO, as is possible for many of the CYPs. Possible exceptions to this rule are trimethylamine or ranitidine N-oxygenation (Lang et al., 1998; Kang et al., 2000; FMO3-selective) and the stereoselective N-oxygenation of nicotine to trans-(*S*)-(−)-nicotine N-1′-oxide (Damani et al., 1988; Cashman et al., 1992a; Park et al., 1993; Berkman et al., 1995; Cashman, 2002).

4.1. Role of flavin-containing monooxygenase in metabolism of N-containing xenobiotics

A number of therapeutic and recreational drugs are substrates for FMO. Table 5 provides a partial listing of nitrogen-containing substrates. Unlike the case with CYP, there are few pharmaceutical agents for which FMO has been shown to be the primary determinant of efficacy and/or toxicity. Tertiary amines, produced by FMO-mediated N-oxygenation, are highly lipophilic, easily excreted, and typically exhibit markedly less pharmacological and/or toxicological properties than the parent amine or CYP-mediated metabolites. For this reason, FMO-mediated N-oxygenation of tertiary amines usually represents detoxication. For example, the neurotoxicant 1-methyl-4- phenyl-1,2,3,6-tetrahydropyridine (MPTP) is readily N-oxygenated by FMO to the N-oxide (Weissman et al., 1985;Cashman, 1986;Cashman & Ziegler, 1986;Chiba et al., 1988,1995;DiMonte et al., 1988;Ottoboni et al., 1990;Wu et al., 1992;Wu & Ichikawa, 1994,1995;Narimatsu et al., 1996), a non-toxic easily excreted metabolite. If not N-oxygenated by FMO, MPTP can undergo CYP and monoamine oxidase-catalyzed metabolism to the potent neurotoxins MPDP+ and MPP+ resulting in parkinsonism. The activity of FMO in the N-oxygenation of MPTP in brain is probably of critical importance and may explain species differences in toxicity (Mushiroda et al., 2001). The contribution of FMO N-oxygenation may be underestimated as the N-oxide can be reduced back to the parent amine by CYP or other reductases. Such is certainly the case for tamoxifen, nicotine, and many other drugs with tertiary amines subject to N-oxygenation. By this mechanism, FMO N-oxygenation provides a reservoir of available parent drug, prolonging its action. It should be emphasized again that aliphatic tertiary amine oxidation by CYP and FMO typically yields distinct products; CYP oxidation results in N-dealkylation, whereas FMO produces exclusively the N-oxide. Aryl amines and primary aryl amines are seldom N-oxygenated by FMO, probably due to electron delocalization into the aromatic ring (Frederick et al., 1982;Hammons et al., 1985;Hlavica et al., 1997). In the case of N-oxygenation of amines in heterocycles to N-oxides, this is almost exclusively catalyzed by CYP.

The contribution of FMO to the metabolism of secondary amines, such as methamphetamine (Yamada et al., 1984; Cashman et al., 1999), may also be underestimated. In this case, it is not reduction back to the parent amine, such as with tertiary amines, but rather the production of metabolites that may be attributed to CYP action. Secondary amines are oxygenated by FMO initially to the *N*-hydroxy amine followed by a second oxygenation to nitrones. The nitrones

are typically rapidly hydrolyzed to yield a hydroxylated primary amine and an aldehyde (Ziegler, 1980, 1988). The hydroxylated primary amine is then reduced enzymatically to the primary amine, producing the same product as CYP-mediated N-dealkylation. Unlike the N-oxygenation of tertiary amines, FMO-mediated metabolism of secondary amines does not always result in detoxication. For example, 3,3′-iminodipropionitrile is a secondary amine that is bioactivated by FMO to the neurotoxic *N*-hydroxy-3,3′iminodipropionitrile (Nace et al., 1997). Another example is *N*-deacetyl ketoconazole which is N-hydroxylated by a number of FMOs (Table 5; Rodriguez & Acosta, 1997a; Rodriguez et al., 1999; Rodriguez & Miranda, 2000). FMO-mediated metabolism of *N*-deacetyl ketoconazole appears to be involved in hepatotoxicity following administration as a antifungal agent (Rodriguez & Acosta, 1997b; Rodriguez & Buckholz, 2003). In addition, evidence exists that FMO-dependent production of hydroxylated secondary (and primary) amines can lead to complexation with hemoproteins, including CYP, resulting in inhibition and perhaps subsequent toxicity (Jonsson & Lindeke, 1992).

Primary alkyl amines can be positive effectors of certain FMO enzymes or serve as substrates for other FMOs. In the latter case, the initial *N*-hydroxylamine is a markedly better substrate than the parent amine and is rapidly oxygenated a second time to give the oxime, often in a stereospecific fashion (e.g., the *trans*-oxime with FMO2; Williams et al., 1984; Tynes et al., 1985, 1986; Poulsen et al., 1986). Oxime formation from primary alkyl amines can thus usually be attributed to FMO. Oximes typically have little pharmacological or toxicological activity and are readily excreted.

Hydrazines can also be excellent substrates for FMO. The initial product observed is the hydrazone, which is subsequently hydrolyzed to form an aldehyde and a dealkylated hydrazine (Prough et al., 1981; Ziegler, 1984, 1988; Gomez & Castro, 1986; Jenner & Timbrell, 1995). Both CYP and FMO are capable of carrying out this reaction; however, it appears that CYP oxygenates 1,2-disubstituted hydrazines, whereas FMO exclusively oxygenates 1,1-disubstituted hydrazines (Ziegler, 1984, 1988). In addition to industrial uses, hydrazines have been used as pharmaceutical agents for a number of years. The use of hydrazines in therapeutics has been limited by toxicity in liver and other organs. The contribution of FMO-mediated oxygenation of 1,1-disubstituted hydrazines to the risk of developing hydrazine toxicities is not known.

It is possible, based on in vitro studies with human hepatocytes or hepatic microsomes, to predict for which N-containing drugs FMO-mediated N-oyxgenation should play a predominant role in pharmacokinetics (Table 5). The nitrogen-containing drugs most likely to be metabolized predominantly, if not exclusively, in vivo by FMO include chlor- and bromo-pheniramine, zimeldine, ranitidine, benzydamine, olopatadine, xanomeline, pargyline, and itopride.

4.2. Role of flavin-containing monooxygenase in metabolism of S-containing xenobiotics

FMO readily catalyzes the S-oxygenation of numerous drugs and xenobiotics (Table 6). These include sulfides, thiols and disulfides, thiocarbamides and thioamides, and mercapto-purines, pyrimidines, and imidazoles (Ziegler, 1980,1982,1988,1991;Poulsen, 1981). Thiols are oxygenated to disulfides through an intermediate sulfenic acid. Disulfides can be further oxygenated to S-oxides (e.g., butyldisulfide S-oxide). The endogenous substrate cysteamine is oxygenated to the disulfide cystamine. Cystamine is not further oxygenated by FMO. Another endogenous substrate, the disulfide lipoic acid, appears to be a better substrate than dihydrolipoamide.

Many therapeutic drugs possess a sulfide pharmacophore. Sulfides are typically excellent substrates for FMO and are readily converted to the sulfoxide (Ziegler, 1980, 1982, 1984;

Poulsen, 1981). The S-oxygenation of thioureas, thioether-containing insecticides such as phorate and disulfoton, and FMO-mediated sulfoxidation of cysteine S-conjugates have already been discussed. FMO readily S-oxygenates simple sulfides such as methyl-, ethyl-, ethyl methyl, 4-chlorophenyl methyl, and diphenyl sulfides (Poulsen & Ziegler, 1995; Nnane & Damani, 2003a, 2003b). Sulfide-containing drugs, for which FMO is active in S-oxygenation to sulfoxides, include cimetidine, rantidine, thioridazine, 7-(1,3-thiazolidin-2-methyl) theophylline, (+)-*cis*-3,5-dimethyl-2-(3-pyridyl)-thiazolidin-4-one, sulindac sulfide, clindamycin, albendazole, and fenbendazole (Eriksson & Bostrom, 1988; Grosa et al., 1992; Cashman et al., 1993b; Lanusse et al., 1993; Moroni et al., 1995a; Nunoya et al., 1995; Poulsen & Ziegler, 1995; Hamman et al., 2000; Rawden et al., 2000; Nnane & Damani, 2003a, 2003b; Wynalda et al., 2003; Virkel et al., 2004). Although not employed as a therapeutic drug, the cyclic sulfide tetrahydrothiophene is the best substrate to date, as evidenced by sub-micromolar K_m values, found by us with FMO. FMO can catalyze the further oxygenation of the sulfoxide to the sulfone, but often this reaction is more efficiently catalyzed by CYP. In the case of chiral sulfides, the sulfoxygenation is often stereoselective (Cashman & Williams, 1990; Rettie et al., 1990, 1994, 1995; Cashman et al., 1993b; Kashiyama et al., 1994; Buronfosse et al., 1995; Moroni et al., 1995a; Hamman et al., 2000). Interestingly, this stereoselectivity is often distinct from that of CYP sulfoxygenation of the same achiral substrate.

Related to the thioureas, or thiocarbamides, are the thioamides such as thioacetamide and thiobenzamide. Thioacetamide and thiobenzamide are potent liver toxicants (Hunter et al., 1977; Hanzlik et al., 1980; Grossman et al., 1991), and the hepatotoxicity appears primarily due to FMO-mediated S-oxygenation to sulfines and sulfenes (Cashman & Hanzlik, 1981; Dyroff & Neal, 1983; Chieli & Malvaldi, 1984; Ziegler, 1984; Ruse & Waring, 1991; Lee et al., 2003).

Ethionamide (2-ethyl-4-thiocarbamoylpyridine) is structurally related to thiobenzamide and is being utilized in the treatment of tuberculosis. Recently, the laboratory of Dr. Paul Ortiz de Montellano demonstrated that ethionamide is a prodrug that must be S-oxygenated by an FMO in the target organism, *Mycobacterium tuberculosis*, to exert its effect (Vannelli et al., 2002). The metabolic activation of ethionamide by the bacterial FMO is the same as the mammalian FMO activation of thiobenzamide to produce hepatotoxic sulfinic and sulfinic acid metabolites. Not surprisingly, Dr. Ortiz de Montellano's laboratory and our own have found ethionamide to be a substrate for human FMO1, FMO2, and FMO3 (unpublished observations). Approximately 25% of individuals have to discontinue ethionamide due to toxicity, primarily in liver, thought to be due to FMO3-dependent S-oxygenation. It would be of interest to determine if particular FMO3 allelic variants correlated to susceptibility to ethionamide hepatotoxicity.

Sulfur-containing drugs for which FMO may be the primary determinant of metabolism in vivo include cimetidine, albendazole, sulindac sulfide, 7-(1,3-thiazolidin-2- ylmethyl) theophylline, (+)-*cis*-3,5-dimethyl-2-(3-pyridyl)- thiazolidin-4-one hydrochloride (SM-12502), methimazole, and ethionamide (Table 6). Xenobiotics with "soft nucleophile" substituents other than nitrogen and sulfur, can also be FMO substrates (Table 7).

5. Conclusions and suggestions for future research

The flavin-containing monooxygenase (FMO) is a family of enzymes that like the cytochrome *P*450 (CYP) superfamily evolved during plant–animal warfare to metabolize a plethora of xenobiotics. There are numerous similarities between these monooxygenase systems. Both utilize reducing equivalents from NADPH to reduce the second atom of molecular oxygen to water and incorporate the other atom into the substrate. FMO and CYP are between 50 and 60

kDa, located in the endoplasmic reticulum and are present in highest amounts in liver with significant amounts also present in other portals of entry for xenobiotics or in excretory organs (nasal, lung, GI, skin, kidney). CYP and FMO are active toward many of the same xenobiotics. There are, however, numerous differences. Unlike the CYP gene superfamily, FMO has relatively few genes (11 in humans, 6 of which are pseudogenes). FMO is a flavoprotein with a single tightly but not covalently-bound FAD. The catalytic cycle for FMO is markedly distinct from CYP, the major differences being that with FMO there is no real substrate binding and electrons are transferred in a single 2-electron step. Although FMO and CYP share many of the same substrates, the products are usually distinct, e.g., N-oxides from tertiary amines rather than the N-dealkylation seen with CYPs. In the case of chiral compounds, the stereoselectivity is often distinct. There are no identified mechanism-based (suicide substrate) inhibitors of FMO, and polyclonal antibodies inhibit catalytic activity weakly, if at all. Many of the CYP families are involved in endobiotic synthesis or catabolism pathways, but the “physiological function” of FMOs, other than in xenobiotic metabolism is uncertain. One theory already discussed and supported by the properties of the yeast FMO is that mammalian FMO can function in control of the cellular redox state. Most FMOs tend to be inhibited by preincubation at 45–50 °C in the absence of NADPH or by anionic detergents, conditions which do not affect CYP activity. FMOs, like CYPs, exhibit tissue- and developmental-dependent expression, but are not inducible by xenobiotics as are the CYPs. In part, owing to the relatively fewer number of FMOs compared to the drug-metabolizing CYPs, there are fewer examples of significant effects of FMO genetic polymorphisms or inhibition on adverse drug–drug interactions leading to clinical failures of therapy or to toxicity. In drug development, however, this may be an advantage, especially given that, with the exception of the thioamides, thiocarbamides, and thiocarbamates where FMO oxygenation of sulfur results in bio-activation, FMO produces relatively fewer toxic metabolites than CYP. The contribution of FMOs in the metabolism of drugs and other xenobiotics is still an incomplete picture in many cases. Relative to the CYPs, for most, but not all, drugs, FMOs probably play a secondary role. However, in some cases (see Tables 5 and 6), FMO activity may predominate. Further efforts to understand the role of FMO in drug metabolism and the potential for incorporation of such information in development of new drugs are important areas. Another critical area of future research is in furthering our knowledge of the structure/function of FMOs. To date, unlike CYPs, there is only a single potential bacterial (PAMO) and no mammalian X-ray structures upon which to model the FMO protein. As a result, many unanswered questions remain including the dimensions of the substrate access channel and the mechanism by which many endogenous nucleophiles are excluded, differences between FMO enzymes that result in exclusion of certain bulkier substrates, access channels for O_2 and NADPH to the FAD, determinations of FMO stereospecificity, and explanations for how the C4a hydroperoxy FAD (FADOOH) is stabilized and the protein is protected from oxidative damage from reactive oxygen “leakage”. The structure of bacterial analogues such as PAMO, or the engineering of a more soluble mammalian FMO, amendable to crystallization, may in the future enable researchers to address these and other questions on FMO structure/function. Finally, a critical area of future research is in increasing our understanding of how genetic variation impacts individual susceptibility to xenobiotics and therapeutic responses to drugs.

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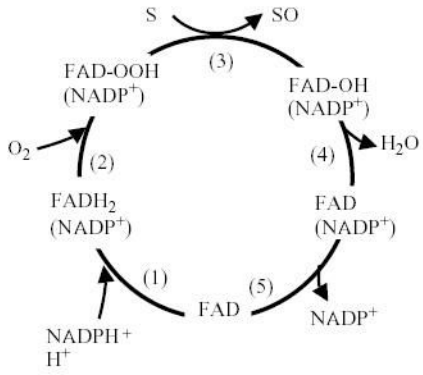

Fig. 1.

Catalytic cycle of flavin-containing monooxygenase (adapted from Ziegler, 1991). (1) FAD reduced by NADPH (fast). (2) $FADH_2$ reacts with O_2 (fast). Flavin-hydroperoxide is stable: thought to be the form in which FMO exists in the cell. (3) FAD-OOH reacts with any suitable nucleophile gaining access to active site. No substrate binding required. (4) One atom of O_2 is incorporated into substrate and the other into H_2O-FMO is a monooxygenase. (5) FAD-OH is converted to FAD via release of H_2O (slowest step in the cycle determines V_{max}). The final step in the cycle is the release of $NADP^+$ (slow).

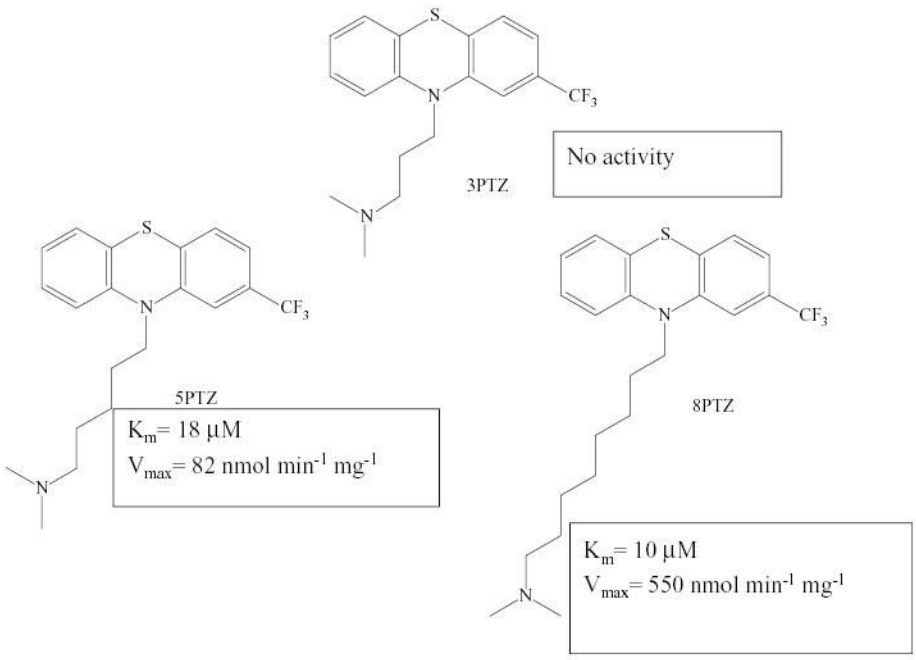

Fig. 2.
Human FMO2 substrate specificity toward phenothiazines with tertiary amine substituents of differing length.

FMO sequence location[b]	Motif number[c]	Sequence of PRINTS motif[d]	Hypothesized structural function[e]
3-26	1, 1a, 1b, (1c)	GXGXXG K***KVAVIGAGVSGLISLK***CCVDEGL	Binding site of the ADP moiety of FAD
27-51	2, 2b, (2c, partial)	D EXXXXXGG *EPTCFERTED*IGGLWRFKENVEDGR	FAD domain, GG stabilizes FAD binding
55-77	3	YQSVITNTSKEMSCFSDFPMPED	*Dimer loop, possible membrane interaction*
79-94	4	PNFLHNSKLLEYFRIF	
104-116	5	IQFQTTVISVKKR	*Isoform specific FAD binding sheet*
124-140	6	QWEVVTQSNSKQQSAVF	
140-156	7, (2a), (3c)	DXXXXATG FDA**VMVCSGHHIL**PNIP	Linkage between FAD and NADPH binding domains
166-185	8	FXGXXXHXXXY FKGQY FHSRQYKHPAGLEGK	Involved with NAD(P)H binding
186-213	9, 3a, (4b), 4c	A D GXGXXG E ***RILVIGIGNSAS****DIAV***ELSKKAAQVY**IS**	Binding site of the ADP moiety of NADPH
296-313	10	TIKVKRRVKELTESAAIF	*Isoform-specific swallowtail meander*
315-342	11, (4a), 5c	D EXXXXATG DGTVEEDI**DVIVFATGYTFAFPF**LEESL	Linkage between NADPH and active site domains
363-380	12, (5a), (6c)	GD KST**FACLGLIQ**PLGSIFP	*Binding site for FAD flavin moiety*
380-393	13	PTVELQARWATRVF	*FAD domain side of substrate cleft-isoalloxazine ring*
395-431		(no PRINTS motif)	*FMO isoform-dependent sequence of substrate cleft*
441-455		(no PRINTS motif)	Active site to active site linkage of monomers
473-509		(no PRINTS motif)	*Dimer interface and active site of substrate cleft*
510-535		(no PRINTS motif)	Non-essential sequence, may interact with membrane

[a]Table has been adapted and updated from Ziegler and Poulsen (1998) to reflect motifs recognized in release 37 of the PRINTS database (Attwood et al., 2003).
[b]The sequence location is relative to rabbit FMO2 (Accession M32029; Lawton et al., 1990) used as the reference sequence for Fingerprint analysis.
[c]The sequence shown for the given motif is from rabbit FMO2, the exact sequence for other FMOs can be obtained from an FMO sequence alignment. Motif numbers not followed by a lowercase letter indicate that the motif is a FMOXYGENASE motif. Numbers followed by a lowercase "a" indicate recognized homology with a FADPNR motif. Numbers followed by a lowercase "b" indicate recognized homology with an ADXRDTASE motif. Numbers followed by a lowercase "c" indicate recognized homology with a PNDRDTASEI motif. If the motif number is in parentheses, assignment was made by manual comparison with assigned motifs of relatives.
[d]FMOXYGENASE motifs are in black. Motifs of related proteins are in black, unless they alter the length of the FMOOXYGENASE print, in which case the added length is enclosed in a single-lined box (PRINTS relative). A double-lined box indicates sequence added to accommodate the highly conserved mammalian FMO motif identified by Fraaije et al., 2002; the sequence of this motif is enclosed in a double-lined box above FMOXYGENASE motif 8. FADPNR motifs are boldface. ADXRDTASE motifs are italicized. PNDRDTASEI motifs are underlined. Sequences in black text occurring above the indicated motif have been recognized as being conserved by proteins with dinucleotide binding motifs (Vallon, 2000).
[e]If structural evidence is available from crystallized relatives of FMO, the function is stated in plain text. If the function is postulated it is italicized.

Table 2.
Postulated function of conserved sequence motifs[a]

Table 1

Mammalian FMOs purified or expressed

Species	FMO	Purified or expressed	Reference
FMO1			
Dog	1	Expressed—baculovirus	Stevens et al., 2003
Human	1 (wild-type) plus four allelic variants	Expressed—baculovirus	Furnes & Schlenk, 2004
Human	1	Expressed—baculovirus	Yeung et al., 2000
Mouse	1, 3[a]	Purified—kidney	Venkatesh et al., 1991a, 1991b
Mouse	Liver	Purified	Smyser et al., 1985
Mouse	1 and 5[a]	Expressed—*E. coli*	Cherrington et al., 1998
Pig	1	Purified	Ziegler & Mitchell, 1972
Pig	1	Expressed—*E. coli*	Lomri et al., 1993
Rabbit	1, 2, 3, 5	*E. coli*	Rettie et al., 1994
Rabbit	1	Expressed—Cos-1 cells	Lawton et al., 1991
Rabbit	1, 3	Purified—liver	Ozols, 1991, 1994
Rat	1	Expressed—yeast	Chiba et al., 1995
Rat	1 and 3[a]	Purified—liver	Krause et al., 1996
Rat	1	Expressed—yeast	Itoh et al., 1993
Rat		Purified—liver	Kimura et al., 1983
Sheep	1, 3[a]	Purified—liver	Longin-Sauvageon et al., 1998
FMO2			
Human	2	Expressed—baculovirus	Dolphin et al., 1998
Human	2 (wild-type) plus five allelic variants	Expressed—baculovirus	Krueger et al., 2002a, in press
Monkey	2	Expressed—baculovirus	Krueger et al., 2001
Mouse		Purified—lung and liver	Tynes et al., 1985
Mouse	2	Expressed—*E. coli*	Karoly & Rose, 2001
Mouse	Lung	Purified	Venkatesh et al., 1992
Rabbit	2	Purified—lung	Williams et al., 1984, 1985
Rabbit	2	Purified—lung and liver	Tynes et al., 1985
Rabbit	2	Expressed—COS-1 cells	Lawton et al., 1991
Rabbit	2	Expressed—baculovirus	Krueger et al., 2001
Rabbit	3 (1C1)	Expressed—*E. coli*	Atta-Asafo-Adjei et al., 1993
Rat	2[b]	Expressed—*E. coli*	Lattard et al., 2002
FMO3			
Human	3	Expressed—*E. coli* fusion	Brunelle et al., 1997
Human	3	Expressed—baculovirus	Haining et al., 1997
Human	3 (wild-type) plus three allelic variants	Expressed—baculovirus	Furnes & Schlenk, 2004
Monkey	3	Purified—liver	Sadeque et al., 1993
Monkey	3	Purified—liver	Rettie et al., 1995
Mouse	3	Expressed—*E. coli*	Falls et al., 1997
Rat	3	Purified—liver	Moroni et al., 1995b
FMO4 and FMO5			
Human	4 (modified)	Expressed—*E. coli*	Itagaki et al., 1996
Human	?	Purified—brain	Bhagwat et al., 1996a
Guinea Pig	5	Expressed—*E. coli*	Overby et al., 1995
Human	5	Expressed—*E. coli*	Overby et al., 1995

[a] FMO enzyme identity not definitively established.

[b] Rat FMO2 is truncated and enzymatically inactive.

Table 3

Endogenous FMO substrates

Substrate	FMO	K_m (μM)	Product	Reference
Sulfur-containing				
Cysteamine	pFMO1[a]	120	Disulfide	Poulsen, 1981
	hFMO2	175		Unpublished
S-cysteine conjugates	pFMO1	344–1538	S-oxide (diastereometric excess 10–78%)	Park et al., 1992
S-allyl-L-cysteine	Rat in vivo		Sulfoxide	Krause et al., 2002
S-farnesylcysteine and	pFMO1			Park et al., 1994
S-farnesylcysteine methyl ester		23–34	S-oxide	Park et al., 1994
Dihydrolipoic acid and	pFMO1	1700		Taylor & Ziegler, 1987
Lipoic acid	pFMO1	120		Taylor & Ziegler, 1987
	hFMO2	71		Unpublished
Methionine	hFMO3	20,000	(D>L)-sulfoxide	Ripp et al., 1999
	rat FMO3	3400	(D>L)-sulfoxide	Krause et al., 1996
	Rabbit FMO1	48,000	(D/L=1.5)-sulfoxide	Duescher et al., 1994
	Rabbit FMO2	30,000	(D/L=0.7)-sulfoxide	
	Rabbit FMO3	6500	(D/L=8)-sulfoxide	
Nitrogen-containing				
Trimethylamine	hFMO3	28	N-oxide	Lang et al., 1998
	pFMO1	617		Sabourin & Hodgson, 1984
	Mouse liver FMO	2340		Sabourin & Hodgson, 1984

[a] p = Pig; h = human.

Table 4

Enzymatic activity of human FMO1, FMO2 and FMO3 allelic variants

Variant	Substrate	K_m (μM)	V_{max} (min^{-1})	Reference
FMO1				
Wild type	Methimazole	7	64	Furnes & Schlenk, 2004
p.H97Q		14	104	
p.I303V		7	71	
p.I303T		16	113	
p.R502X		—	—	
Wild type	Imipramine	14	51	
p.H97Q		15	66	
p.I303V		20	48	
p.I303T		14	71	
p.R502X		16	25	
Wild type	Fenthion	340	93	
p.H97Q		320	125	
p.I303V		351	99	
p.I303T		240	149	
p.R502X		300	51	
Wild-type	Methyl *p*-tolyl sulfide	284	46	
p.H97Q		251	66	
p.I303V		300	42	
p.I303T		245	84	
p.R502X		200	32	
Note: Some of the variants reported above may be rare alleles. See Hines et al. (2003) for a description of a common allelic variant (*FMO1*6*) that effects transcription levels.				
FMO2				
Wild type	Ethylenethiourea	16	41	Krueger et al., in press
p.N413K		40	124	Krueger et al., in press
FMO variant alleles with no demonstrable activity toward ethylenethiourea include p.71D dup, p.V113X, p.S1195L, p.X472.				Krueger et al., in review
FMO3				
Wild type	Fenthion	145	11	Furnes & Schlenk, 2004
p.D132H		150	10	Furnes & Schlenk, 2004
p.E158K		200	12	Furnes & Schlenk, 2004
Wild type	5-DPT	155	51	Cashman et al., 2000
		929	65	Lattard et al., 2003a, 2003b
p.E158K		187	44	Cashman et al., 2000
		763	20	Lattard et al., 2003a, 2003b
p.V257M		82	13	Cashman et al., 2000
p.F510X		62	0.1	Cashman et al., 2000
p.E132H		562	37	Lattard et al., 2003a, 2003b
p.E132H/E158K		1517	41	Lattard et al., 2003a, 2003b
p.E308G		2280	33	Lattard et al., 2003a, 2003b
p.E158K/E308G		2285	26	Lattard et al., 2003a, 2003b
p.L360P		993	130	Lattard et al., 2003a, 2003b
Wild type	Trimethylamine	32	189	Cashman et al., 2000
		8	40	Lattard et al., 2003a, 2003b
p.E158K		206	105	Cashman et al., 2000
		17	30	Lattard et al., 2003a, 2003b
p.V257M		1151	28	Cashman et al., 2000
p.F510X		1034	35	Cashman et al., 2000
p.E132H		26	40	Lattard et al., 2003a, 2003b
p.E132H/E158K		11	20	Lattard et al., 2003a, 2003b
p.E308G		42	18	Lattard et al., 2003a, 2003b
p.E158K/E308G		23	8	Lattard et al., 2003a, 2003b
p.L360P		11	220	Lattard et al., 2003a, 2003b
Wild type	Tyramine	231	110	Cashman et al., 2000
p.E158K		941	106	Cashman et al., 2000
p.V257M		2164	41	Cashman et al., 2000
p.F510X		1384	11	Cashman et al., 2000
Wild type	Methimazole	12	59	Lattard et al., 2003a, 2003b
p.E158K		10	27	Lattard et al., 2003a, 2003b
p.E132H		15	48	Lattard et al., 2003a, 2003b
Wild type	Methimazole	12	59	Lattard et al., 2003a, 2003b
p.E132H/E158K		10	19	Lattard et al., 2003a, 2003b
p.E308G		17	15	Lattard et al., 2003a, 2003b
p.E158K/E308G		12	11	Lattard et al., 2003a, 2003b
p.L360P		8	184	Lattard et al., 2003a, 2003b
p.N61S			~15	Dolphin et al., 2000
Wild type	Ranitidine	3	27	Park et al., 2002
p.E158K		.1	13	Park et al., 2002
p.E308G		1	13	Park et al., 2002
p.E158K/E308G		0.7	3	Park et al., 2002

Variant alleles with no activity toward 5-DPT, tyramine or trimethylamine include p.M66I, p.P153L, p.E305X (Treacy et al., 1998; Dolphin et al., 1997); p.R492W (Akerman et al., 1999a); p.A52T; p.387L; p.E314X (Akerman et al., 1999b); p.I199T (Zschocke et al., 1999); p.M82T (Murphy et al., 2000); p.E32K (Zhang et al., 2003); p.V143E (Basarab et al., 1999); p.N61S; p.M434I (Dolphin et al., 2000); p.V58I (Kubota et al., 2002); p.D198I; p.R205C (Fujieda et al., 2003). Note: expressed p.N61S is fully active toward methimazole but is incapable of N-oxygenation of trimethylamine (Dolphin et al., 2000).

Table 5

N-containing drugs and xenobiotics oxygenated by FMO[a]

Substrate	FMO	K_m (μM)	K_{cat}	Product	Reference
Primary amines					
2-Aminofluorene	pFMO1[b]	5[b]		*N*-hydroxy	Frederick et al., 1982
Amphetamine	hFMO3			*trans:cis* oxime 5:1	Cashman et al., 1999
m-Amylamine	rFMO2	>50,000			Tynes et al., 1986
n-Heptylamine	rFMO2	27,000			Tynes et al., 1986
N-octylamine	rFMO2	6700			Tynes et al., 1986
N-octylamine	rFMO2	12,000			Poulsen et al., 1986
N-nonylamine	rFMO2	1800			Tynes et al., 1986
N-decylamine	rFMO2	400			Tynes et al., 1986
N-undecylamine	rFMO2	90			Tynes et al., 1986
N-dodecylamine	rFMO2	33			Tynes et al., 1986
N-dodecylamine	rFMO2	290		*cis*-oxime	Poulsen et al., 1986
N-tridecylamine	rFMO2	13			Tynes et al., 1986
C8 and C10 *N*-	hFMO3			*cis*-oximes	Lin et al., 1996
(*n*-octylamino-	hFMO3	90–197		*trans*-oxime	Lin & Cashman, 1997b
phenethylamine)					
2-(Trifluoromethyl)	hFMO3			*cis*-oxime	Lin et al., 1996
phenothiazines					
Tyramine	hFMO3	220–950		*trans*-oxime	Lin & Cashman, 1997a
Secondary amines					
p-Chloro-*N*-methylaniline	pFMO1				Prough & Ziegler, 1977
N-deacetyl ketoconazole	hFMO1 and rFMO1			*N*-hydroxy/nitrone	Rodriguez & Miranda, 2000
	r-FMO2				Rodriguez & Miranda, 2000
	Human and rabbit FMO3				
	pFMO1	35			Rodriguez & Acosta, 1997a, 1997b
Desipramine	pFMO1	250			Ziegler, 1988
Desmethyltrifluperazine	pFMO1	9			Ziegler, 1988
Ephedrine	pFMO1				Ziegler, 1988
N-fluoro-*N*-methylaniline	Rat liver FMO		19	C4-hydroxy	Boersma et al., 1993
	Rat liver FMO		53	*N*-hydroxyl	Boersma et al., 1993
	Rat liver FMO		93	*N*-demethyl	Boersma et al., 1993
3,3′-Iminodipropionitrile	Rat in vivo			*N*-hydroxy	Nace et al., 1997
Methamphetamine	Guinea pig in vitro			Amphetamine	Yamada et al., 1984
	Rat in vitro				
Methamphetamine	hFMO3			Phenylpropanone	Cashman et al., 1999
N-methylaniline	pFMO1	30		*N*-hydroxy	Ziegler, 1988
	Mouse liver FMO	1060			Sabourin & Hodgson, 1984
N-methyloctylamine	rFMO2	120			Poulsen et al., 1986
Nortryptyline	pFMO1	500			Ziegler, 1988
Propranolol	pFMO1	210		*N*-hydroxy	Wu et al., 2004
	pFMO1	400			Ziegler, 1988
Tertiary amines					
ABT-418	pFMO1			100% *trans* N-oxide	Rodrigues et al., 1995
	rFMO2			100% *trans* N-oxide	Rodrigues et al., 1995
	hFMO3			1:1 *cis:trans* N-oxide	Rodrigues et al., 1995
Acetylpromazine	pFMO1	53			Ziegler et al., 1971a, 1971b
Amitriptyline	pFMO1	100		N-oxide	Ziegler, 1980
Benzphetamine	pFMO1	74			Sabourin & Hodgson, 1984
	Mouse liver FMO	1670			Sabourin & Hodgson, 1984
	Mouse liver FMO	130		N-oxide	Ziegler, 1988
Benzydamine[c]	*hFMO1*	*60*	*46*	*N-oxide*	Lang & Rettie, 2000
	hFMO1	*24*		*N-oxide*	Stormer et al., 2000
	hFMO3	*80*	*36*		Lang & Rettie, 2000
	hFMO3	*40*			Stormer et al., 2000
Bromdiphenhydramine	pFMO1	47			Ziegler et al., 1971a
Brompheniramine	*pFMO1*				Prough & Ziegler, 1977
	pFMO1				Cashman et al., 1993a
	pFMO1	200		N-oxide	Ziegler, 1988
Bupivacaine	pFMO1				Wu et al., 2004
Butytyriptyline	pFMO1	100			Ziegler et al., 1971a
Chlorimipramine	pFMO1	40			Ziegler et al., 1971a
(D)-Chlorpheniramine	*pFMO1*	*76*		*N-oxide*	Cashman et al., 1992b

Substrate	FMO	K_m (μM)	K_{cat}	Product	Reference
Tertiary amines					
(ι)-Chlorpheniramine	*pFMO1*	*300*		*N-oxide*	Cashman et al., 1992b
Chlorpromazine	pFMO1	9		N-oxide	Sofer & Ziegler, 1978
Chlorprothixene	pFMO1	30			Ziegler et al., 1971a
Clorgyline	pFMO1	2		N-oxide	Wu & Ichikawa, 1995
Clozapine	hFMO3	324		N-oxide	Tugnait et al., 1997
Deprenyl	pFMO1	65		N-oxide	Cashman & Ziegler, 1986
	pFMO1	14			Wu & Ichikawa, 1995
Diethazine	pFMO1	600			Ziegler et al., 1971a
N,*N*-diethylaniline	pFMO1	130			Ziegler et al., 1971a
	Mouse liver FMO	144			Sabourin & Hodgson, 1984
N,*N*-dimethyloctylamine	rFMO2	46			Poulsen et al., 1986
Diphenhydramine	pFMO1	160		N-oxide	Ziegler, 1988
N-ethyl-*N*-methylaniline	pFMO1	59			Ziegler et al., 1971a
	pFMO1	20		N-oxide (*S*/*R*=4)	Hadley et al., 1994
Ethylmorphine	pFMO1	284			Sabourin & Hodgson, 1984
	Mouse liver FMO	1650			Sabourin & Hodgson, 1984
Fluphenazine	pFMO1	12			Sofer & Ziegler, 1978
Guanethidine	pFMO1	310		N-oxide	McManus et al., 1983
	pFMO1	170			Ziegler, 1988
Imipramine	pFMO1	22		N-oxide	Cashman & Ziegler, 1986
	dFMO1	5			Stevens et al., 2003
	Mouse liver FMO	27			Sabourin & Hodgson, 1984
	Human brain			N-oxide	Bhagwat et al., 1996b
Itopride	*hFMO1 and FMO3*			*N-oxide*	Mushiroda et al., 2000
K11777	hFMO3	109		N-oxide	Jacobsen et al., 2000
Lidocaine	pFMO1				Wu et al., 2004
Methaphenilene	pFMO1	42			Ziegler et al., 1971a
Methapyrilene	pFMO1	93			Ziegler et al., 1971a
Methdilazine	pFMO1	55			Ziegler et al., 1971a
Methotrimeprazine	pFMO1	9			Ziegler et al., 1971a
Methoxypromazine	pFMO1	33			Ziegler et al., 1971a
Methyapyrilene	pFMO1	93		N-oxide	Ziegler, 1988
MPTP	rFMO1	2		N-oxide	Chiba et al., 1995
	pFMO1	32		N-oxide	Cashman & Ziegler, 1986
	pFMO1	38		N-oxide	Wu & Ichikawa, 1995
(*S*)-nicotine	Monkey FMO2	1700	0.6	N-oxide	Krueger et al., 2001
	rFMO2	500	7	N-oxide	Krueger et al., 2001
	pFMO		*trans:cis* 57:43	N-oxide	Park et al., 1993
	hFMO3		*trans* only	N-oxide	Park et al., 1993
	rFMO2		*trans* only	N-oxide	Park et al., 1993
	pFMO1	181	37-1:1 *cis:trans*	N-oxide	Damani et al., 1988
	pFMO1	380	57		Williams et al., 1990
	rFMO2	1030	<1		Williams et al., 1990
(*R*)-(+)-nicotine	pFMO1	70	41-*trans*	N-oxide only	Damani et al., 1988
Olopatidine	*hFMO1 and FMO3*			*N-oxide*	Kajita et al., 2002
Pargyline	*pFMO1*	*65*		N-oxide	Cashman & Ziegler, 1986
	pFMO1	12			Wu & Ichikawa, 1995
Phenethiazine	pFMO1	120			Ziegler et al., 1971a
Pheniramine	pFMO1	1300			Ziegler et al., 1971a
Prochlorperazine	pFMO1	3			Sofer & Ziegler, 1978
	rFMO2	3			Poulsen et al., 1986
Promazine	pFMO1	66		N-oxide	Ziegler, 1988
Promethazine	pFMO1	120			Ziegler et al., 1971a
Propiomazine	pFMO1	100			Ziegler et al., 1971a

Substrate	FMO	K_m (μM)	K_{cat}	Product	Reference
Prothipendyl	pFMO1	1			Ziegler et al., 1971a
S 16020	hFMO3			N-oxide	Pichard-Garcia et al., 2004
SnI-2011	hFMO1			N-oxide	Washio et al., 2001
Tamoxifen	Mouse FMO1	1200		N-oxide	Hodgson et al., 2000
	hFMO3	1400		N-oxide	
Thioperazine	pFMO1	11		N-oxide	Sofer & Ziegler, 1978
Thioridazine	Mouse liver			N-oxide	Blake et al., 1995
Tertiary amines					
Trifluoperazine	pFMO1	13			Sofer & Ziegler, 1978
	rFMO2	31			Poulsen et al., 1986
Triflupromzaine	pFMO1	20			Ziegler, 1988
Trimeprazine	pFMO1	20			Ziegler et al., 1971a
Trimethylamine	mFMO2	1686		N-oxide	Karoly & Rose, 2001
Verapamil	pFMO1	64		N-oxide (*S*/*R*=10)	Cashman, 1989
	Rat liver	51		N-oxide (*S*/*R*=7)	Cashman, 1989
Xanomeline	*hFMO1 and FMO3*			*N-oxide*	Ring et al., 1999
Zimeldine	Rat liver			N-oxide	Cashman et al., 1988
	Pig liver			N-oxide	Cashman et al., 1988
Hydrazines					
N-aminohomopiperdine	pFMO1	170			Ziegler, 1988
N-aminomorpholine	pFMO1	610			Ziegler, 1988
N-aminopiperdine	pFMO1	30		Hydrazone	Ziegler, 1988
N-aminopyrrolidine	pFMO1	100			Ziegler, 1988
1,1-Dimethylhydrazine	pFMO1	430			Ziegler, 1988
1,2-Dimethylhydrazine	pFMO1	5600			Ziegler, 1988
1-Methyl-1-phenylhydrazine	pFMO1	80		Hydrazone	Ziegler, 1988
1-Methyl-2-phenylhydrazine	pFMO1	2000			Ziegler, 1988
Monobenzylhydrazine	pFMO1	7000			Ziegler, 1988
Monobutylhydrazine	pFMO1	6900			Ziegler, 1988
Monomethylhdrazine	Rat liver in vitro				Gomez & Castro, 1986
	pFMO1	35,000			Prough, 1973
Monophenylhydrazine	pFMO1	3000			Ziegler, 1988
Procarbazine	pFMO1	1800			Ziegler, 1988

[a] *N*,*N*-dimethyaniline is not included as it has been used by many researchers to assay FMO activity.

[b] p = pig; h = human; d = dog; r= rabbit; m= mouse.

[c] Drugs for which FMO-mediated metabolism may predominate in humans is shown in italics.

Table 6

S-containing drugs and xenobiotics oxygenated by FMO[a]

Substrate	FMO	K_m (μM)	K_{cat} (min^{-1})	Product	Reference
Thiols					
Benzylmercaptan	pFMO1[b]	55			Ziegler, 1980
n-Butylmercaptan	pFMO1	33			Ziegler, 1980
Dithiothreitol	pFMO1	465			Ziegler, 1980
Ethanethiol	pFMO1	58		Disulfide	Poulsen, 1981
n-Heptylmercaptan	pFMO1	15			Ziegler, 1980
β-Mercaptoethanol	pFMO1	2800			Ziegler, 1980
Thiophenol	pFMO1	33			Ziegler, 1980
Thioglycerol	pFMO1	721			Ziegler, 1980
Disulfides					
Butyldisulfide	pFMO1	6		S-oxide	Poulsen, 1981
Dibenyldisulfide	pFMO1	45			Ziegler, 1980
o-Dithiane	pFMO1	<3			Ziegler, 1980
trans-4,5-Dihydroxy-o-dithiane	pFMO1	270		S-oxide	Poulsen, 1981
Sulfides/thioethers					
Albendazole[c]	*Rat liver FMO*			*Sulfoxide*	Moroni et al., 1995a
Aldicarb	mFMO liver	607			Usmani et al., 2004
	pFMO1	312			Usmani et al., 2004
2-Aryl-1,3-dithiolanes	rFMO2			*trans*-S-oxides	Cashman & Williams, 1990
(+)-4-Bromophenyl-1,3-oxathiolane	pFMO1			100% *trans*-S-oxide	Cashman et al., 1995
(−)-4-Bromophenyl-1,3-oxathiolane	pFMO1			24% *trans*-S-oxide	Cashman et al., 1995
Benzyl methyl sulfide	pFMO1	1	49	Sulfoxide	Light et al., 1982
4-Chlorophenyl allyl sulfide	pFMO1		53	Sulfoxide	Light et al., 1982
4-Chlorophenyl methyl sulfide	pFMO1	185			Nnane & Damani, 2003b
Cimetidine	*pFMO1*			*S-oxide*	Cashman et al., 1993b
Dazomet	mFMO liver	399			Usmani et al., 2004
	pFMO1	244			Usmani et al., 2004
Demeton-S	mFMO liver	110			Levi & Hodgson, 1988
	pFMO1	40			Levi & Hodgson, 1988
Demeton-O	mFMO liver	59			Levi & Hodgson, 1988
Dimethylsulfide	pFMO1	<3		DMSO	Poulsen, 1981
	Mouse liver FMO	34			Sabourin & Hodgson, 1984
DMSO	pFMO1	90,000		S-dioxide	Poulsen, 1981
Diphenyl sulfide	pFMO1	68			Nnane & Damani, 2003a, 2003b
	pFMO1	74		Sulfoxide	Poulsen, 1981
Disulfoton	mFMO liver	3			Levi & Hodgson, 1988
	pFMO1	2			Levi & Hodgson, 1988
	rFMO2	71	44	Sulfoxide	Henderson et al., 2004a
	hFMO2	32	20	Sulfoxide	Henderson et al., 2004a
	hFMO1	7	37	Sulfoxide	Usmani et al., 2004
Ethylene sulfide	pFMO1	60			Ziegler, 1980
Ethyl methyl sulfide	pFMO1	1380		Sulfoxide	Nnane & Damani, 2003a, 2003b
Fenthion	mFMO liver	12			Levi & Hodgson, 1988
	pFMO1	9			Levi & Hodgson, 1988
	pFMO1	18			Levi & Hodgson, 1988
Flosequinan sulfide	ratFMO1	33		*R*(+)-sulfoxide	Kashiyama et al., 1994
Fonofos	pFMO1	33			Smyser & Hodgson, 1985
Metam-sodium	mFMO liver	572			Usmani et al., 2004
	pFMO1	175			Usmani et al., 2004

Substrate	FMO	K_m (μM)	K_{cat} (min^{-1})	Product	Reference
Methiocarb	mFMO liver	130			Usmani et al., 2004
	pFMO1	115			Usmani et al., 2004
	hFMO1	30	86	Sulfoxide	Usmani et al., 2004
4-Methoxyphenyl allyl sulfide	pFMO1		60	Sulfoxide	Light et al., 1982
2-Methyl-1,3-benzodithiol	pFMO1			100% *cis* (−)-(1*S*,2*R*)	Cashman et al., 1995
S-methyl-*N*-*N*-dithiocarbamate	hFMO1		7.6	S-oxide	Pike et al., 2001
S-methyl-esonarimod	hFMO1,3,5	2.71 (FMO5)		Sulfoxide	Ohmi et al., 2003
Sulfides/thioethers					
Methylphenylsulfide	pFMO1	13			Ziegler, 1980
	pFMO1	16	62	Sulfoxide	Light et al., 1982
	Mouse liver FMO	6			Sabourin & Hodgson, 1984
MK-0767 methyl sulfide	hFMO1,3,5	FMO1 99% stereoselective ee		Sulfoxide	Karanam et al., 2004
4-Nitrophenyl allyl sulfide	pFMO1		53	Sulfoxide	Light et al., 1982
Phenothiazine	pFMO1	12			Nagata et al., 1990
S-phenyl diethyl-phosphinothiolothionate	pFMO1	48			Smyser & Hodgson, 1985
Phorate	mFMO liver	32	80	(−) Sulfoxide	Levi & Hodgson, 1988
	pFMO1	12			Levi & Hodgson, 1988
	hFMO1	49	26	Sulfoxide	Usmani et al., 2004
	rFMO2	71	39	Sulfoxide	Henderson et al., 2004a
	hFMO2	57	41	Sulfoxide	Henderson et al., 2004a
	mFMO2	107		Sulfoxide	Karoly & Rose, 2001
Phorate oxon	mFMO liver	462			Levi & Hodgson, 1988
	pFMO1	256			Levi & Hodgson, 1988
SM-12502	*rat FMO1*			*S-oxide*	Nunoya et al., 1995
Sodium dimethyldithio-carbamate	mFMO liver		761		Usmani et al., 2004
	pFMO1		1360		Usmani et al., 2004
Sodium diethyldithio-carbamate	mFMO liver		739		Usmani et al., 2004
	pFMO1		1099		Usmani et al., 2004
Sulindac sulfide	*rFMO2, hFMO3*			*(R)-sulfoxide*	Hamman et al., 2000
	pFMO1	*3*		*80>90% (R)-sulfoxide*	Light et al., 1982
Sulprofos	mFMO liver	1			Levi & Hodgson, 1988
	pFMO1	1			Levi & Hodgson, 1988
	hFMO1	14	16	Sulfoxide	Usmani et al., 2004
Tazarotenic acid	hFMO1,3			Sulfoxide	Attar et al., 2003
Thiobenzamide sulfoxide	pFMO1	250		S-dioxide	Poulsen, 1981
Thiofanox	mFMO liver	575			Usmani et al., 2004
	pFMO1	1307			Usmani et al., 2004
p-Tolyl methyl sulfide	hFMO1			(*R*) sulfoxide	Yeung et al., 2000
	hFMO1	87			Stevens et al., 2003
	dFMO1	99			Stevens et al., 2003
	hFMO2		63	(*R*)-sulfoxide	Unpublished
p-Tolyl ethyl sulfide	pFMO1	13	48	95% (*R*)-sulfoxide	Light et al., 1982
p-Tolyl substituted sulfides (methyl-*n*-butyl)	rFMO3	<10	100% (*R*) sulfoxide decreasing slightly with chain length		Rettie et al., 1994
	rFMO3	<10	100% (*R*) sulfoxide decreasing markedly with chain length		Rettie et al., 1994
	rFMO3	100–280	90% (*R*) with *n*-butyl-decreasing to no stereoselectivity with methyl or ethyl		Rettie et al., 1994
	hFMO3		80–90% (*R*) for propyl and butyl		Haining et al., 1997
	rFMO5		97–98% (*S*) with methyl and ethyl Little or no activity with propyl		Fisher et al., 1995
Straight-chain and branched alkyl *p*-tolyl sulfides	rFMO2		Stereoselectivity of sulfoxide function of steric factors		Rettie et al., 1990, 1995

Substrate	FMO	K_m (μM)	K_{cat} (min^{-1})	Product	Reference
	Monkey FMO3, rFMO1 and rFMO3		Primarily (*R*)-sulfoxide		Rettie et al., 1995
Methy–hexyl 2-naphthyl sulfides	rFMO2		<0.2–66 97–98% (*R*)-sulfoxide		Fisher & Rettie, 1997
	rFMO1		1–37 83–92% (*R*)-sulfoxide		Fisher & Rettie, 1997
	rFMO5		>92% (*S*)-sulfoxide with methyl		Fisher et al., 1995
	rFMO5		>85% (*S*) with ethyl		Fisher et al., 1995
	rFMO5		Little or no activity with propyl		Fisher et al., 1995
Thiocarbamides and related					
Ethionamide	hFMO2	107	9	Sulfoxide	Unpublished
	hFMO3	184	33	Sulfoxide	Unpublished
Ethylene thiourea	hFMO2	14	50		Krueger et al., 2002a, 2002b
	rFMO2	21	149		Henderson et al., 2004b
α-Naphthylthiourea	pFMO1	4			Nagata et al., 1990
	rFMO2	25	60		Henderson et al., 2004b
	hFMO2	42	33	Sulfoxide	Henderson et al., 2004b
Thiocarbamides and related					
1-Phenylthiourea	pFMO1	4			Poulsen et al., 1979
	hFMO1	3			Henderson et al., 2004b
	rFMO2	82	327		Henderson et al., 2004b
	hFMO2	29	24	Sulfoxide	Henderson et al., 2004b
Thioacetamide	pFMO1	65		S-oxide	Ziegler, 1980
Thioacetanilide	hFMO2				Krueger et al., 2002a
Thiobenzamide	hFMO2				Krueger et al., 2002a
Thiocarbanilide	pFMO1	7			Poulsen et al., 1979
Thionicotinamide	pFMO1	460		S-oxide	Ziegler, 1980
Thiourea	pFMO1	23			Nagata et al., 1990
	hFMO1	4			Kim & Ziegler, 2000
	rFMO2	36	134		Henderson et al., 2004b
	hFMO2	27	51		Henderson et al., 2004b
	mFMO2	11			Karoly & Rose, 2001
	pFMO1	34			Poulsen et al., 1979
Sulfenic acids					
Benzimidazoline-2-thiones	pFMO1			Sulfenic/ sulfinic acids	Decker et al., 1992
Deacetylated spironolactone	pFMO1			Sulfenic acid	Decker et al., 1991
Diethylcarbodithioic acid	pFMO1	75			Poulsen, 1981
1,3-Diphenylformadidine-sulfenic acid	pFMO1	7			Ziegler, 1980
Dithiobenzoic acids and esters	pFMO1	3–7500			Taylor & Ziegler, 1987
Ethylene formadidine–sulfenic acid	pFMO1	190			Ziegler, 1980
Formamidinesulfenic acid	pFMO1	140			Ziegler, 1980
2-Mercaptobenzimidazole	pFMO1	13			Poulsen, 1981
Mercaptobenzoic acid	pFMO1	67			Taylor & Ziegler, 1987
N-methylimidazole-2-sulfenic acid	pFMO1	22		Sulfinic acid	Ziegler, 1980
Phenylcarbodithioic acid	pFMO1	<3			Poulsen, 1981
Phenylformamidine–sulfenic acid	pFMO1	34		Sulfinic acid	Ziegler, 1980
Propylthiouracil	pFMO1	950			Poulsen, 1981
Thiolacetic acid	pFMO1	200			Taylor & Ziegler, 1987

[a] Methimazole is not included as it has been used by many investigators to assay FMO activity.

[b] p = pig; h = human; m = mouse; r = rabbit; d = dog.

[c] Drugs for which FMO-mediated metabolism may predominate in humans is shown in italics.

Table 7

FMO activities not involving S- or N-oxygenation

Substrate	Product	FMO	K_m (μM)	Reference
Carbon				
5,6-Dimethylxanthenone-4-acetic acid	6-Methyl hydroxyl	hFMO3[a]		Zhou et al., 2002
Salicylaldehyde	Pyrocatechol/formate	pFMO1	500–600	Chen et al., 1995
Selenium				
Benzeneselenol		pFMO1	18	Ziegler et al., 1992
Benzylselenide		pFMO1	0.7	Chen & Ziegler, 1994
Dimethylselenide	Selenoxide	pFMO1		Goeger & Ganther, 1994
Methylphenylselenide	Selenoxide	pFMO1	0.3	Chen & Ziegler, 1994
2-(Methylseleno)benanilide	Selenoxide	pFMO1	5	Ziegler et al., 1992
2-(Methylseleno)cinnamonitrile		pFMO1	3	Ziegler et al., 1992
N-[2-(methylseleno)ethyl]benzamide		pFMO1	1	Ziegler et al., 1992
Phenylselenomethyl)trimethylsilane		pFMO1	10	Ziegler et al., 1992
2-Selenylbenzanilide		pFMO1		Chen & Ziegler, 1994
Selenocyteine Se-conjugates	Selenoxide	pFMO1		Rooseboom et al., 2001
Selenourea		pFMO1	3	Ziegler et al., 1992
Others				
Diphenylmethylphosphine	Oxide	pFMO1		Smyser & Hodgson, 1985
Boronic acids		pFMO1		Jones & Ballou, 1986
Iodine		pFMO1		Ziegler et al., 1980

[a] p = pig; h = human.