

Do Cytochrome P450 Enzymes Contribute to the Metabolism of Xenobiotics in Human?

Khaled Abass^{1*}, Petri Reponen^{1,2}, Miia Turpeinen¹,
Sampo Mattila² and Olavi Pelkonen¹

¹*Pharmacology and Toxicology Unit, Institute of Biomedicine, University of Oulu*

²*Department of Chemistry, University of Oulu
Finland*

1. Introduction

The cytochromes P450 (CYP) comprise a large multigene family of hemethiolate proteins which are of considerable importance in the metabolism of xenobiotics and endobiotics. CYP enzymes in humans as well as in other species have been intensively studied during recent years (Pelkonen et al., 2008; Turpeinen et al., 2007). It is possible to characterize metabolic reactions and routes, metabolic interactions, and to assign which CYP is involved in the metabolism of a certain xenobiotic by different *in vitro* approaches (Pelkonen et al., 2005; Pelkonen & Raunio, 2005; Hodgson and Rose, 2007a). Risk assessment needs reliable scientific information and one source of information is the characterization of the metabolic fate and toxicokinetics of compounds. Toxicokinetics refers to the movement of a xenobiotic into, through, and out of the body and is divided into several processes including absorption, distribution, metabolism, and excretion (ADME). Metabolism is one of the most important factors that can affect the overall toxic profile of a pesticide. During metabolism, the chemical is first biotransformed by phase I enzymes, usually by the cytochrome P450 (CYP) enzyme system, and then conjugated to a more soluble and excretable form by phase II conjugating enzyme systems (Guengerich & Shimada, 1991). In general, these enzymatic reactions are beneficial in that they help eliminate foreign compounds. Sometimes, however, these enzymes transform an otherwise harmless substance into a reactive form – a phenomenon known as metabolic activation (Guengerich & Shimada, 1991). Exposure to pesticides is a global challenge to risk assessment (Alavanja et al., 2004; Maroni et al., 2006). On a world-wide basis, acute pesticide poisoning is an important cause of morbidity and mortality. In an extrapolation, WHO/UNEP estimated that more than 3 million people were hospitalized for pesticide poisoning every year and that 220 000 died; it particularly noted that two-thirds of hospitalizations and the majority of deaths were attributable to intentional self-poisoning rather than to occupational or accidental poisoning (Konradsen et al., 2005; WHO/UNEP, 1990). Humans are inevitably exposed to pesticides in a variety of ways: at different dose levels and for varying periods of time (Boobis et al., 2008; Ellenhorns et al., 1997).

* corresponding author email: khaled.megahed@oulu.fi; khaled.m.abass@gmail.com

2. CYPs - the human xenobiotic-metabolizing enzymes

CYPs are found in high concentrations in the liver, but are present in a variety of other tissues, including lungs (Lawton et al., 1990), kidneys ((Hjelle et al., 1986; Tremaine et al., 1985), the gastrointestinal tract (Dutcher & Boyd, 1979; Peters and Kremers, 1989), nasal mucosa (Adams et al., 1991; Eriksson and Brittebo, 1991), skin (Khan et al., 1989) and brain tissue (Bergh & Strobel, 1992; Dhawan et al., 1990). CYPs are categorized into families and subfamilies by their sequence similarities. Humans have 18 families of cytochrome P450 genes and 44 subfamilies. The enzymes are thus identified by a number denoting the family, a letter denoting the subfamily and a number identifying the specific member of the subfamily. The example given below explains the system of nomenclature followed (Fig. 1).

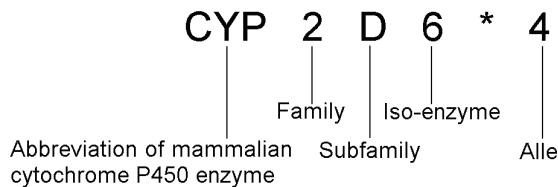


Fig. 1. An example of the nomenclature of the cytochrome P450 enzymes (modified from (Wijnen et al., 2007)).

The website <http://drnelson.utmem.edu/CytochromeP450.html> contains more detailed classification related to the cytochrome P450 metabolizing enzymes. The CYP enzymes in families 1-3 (Fig. 2) are active in the metabolism of a wide variety of xenobiotics including drugs (Pelkonen et al., 2008; Pelkonen et al., 2005; Rendic & Di Carlo, 1997).



Fig. 2. Relative abundance of individual CYP forms in the liver (modified from (Pelkonen et al., 2008)).

2.1 CYP1A subfamily

CYP1A1 and CYP1A2 are members of the CYP1A subfamily. CYP1A1 is a major extrahepatic CYP enzyme and its level of expression in human liver is very low (Pelkonen et al., 2008; Guengerich & Shimada, 1991; Ding & Kaminsky, 2003; Edwards et al., 1998; McKinnon et al., 1991; Pasanen & Pelkonen, 1994; Raunio et al., 1995). In humans, CYP1A2 is expressed mainly in liver and in lower levels in lung along with CYP1A1 (Liu et al., 2003; Wei et al., 2001; Wei et al., 2002). CYP1A2 represents about 10% of total CYP enzymes in the human liver (Pelkonen & Breimer, 1994; Shimada et al., 1994). CYP1A2 enzyme levels in the human liver display some variability between individuals (Shimada et al., 1994).

2.2 CYP2A subfamily

The human CYP2A subfamily contains three genes i.e. CYP2A6, CYP2A7, and CYP2A13, and two pseudogenes (Hoffman et al., 1995; Honkakoski & Negishi, 1997; Pedro et al., 1995).

CYP2A6 represents 10% of the total CYP content in liver (Pelkonen et al., 2008; Yun et al., 1991). CYP2A6 enzyme activity in the human liver displays a relatively large variability between individuals, and some Japanese are known to lack the functional protein completely (Pelkonen et al., 2008; Pelkonen et al., 2000; Shimada et al., 1996).

2.3 CYP2B subfamily

CYP2B6 represents approximately 1-10% of the total hepatic CYPs. A notable interindividual variability in the expression of CYP2B6 has been reported (Code et al., 1997; Fauchette et al., 2000; Lang et al., 2001; Stresser & Kupfer, 1999; Yamano et al., 1989). CYP2B6 has a high polymorphic expression and it is affected by genotype and gender.

2.4 CYP2C subfamily

The CYP2C subfamily has four active members, namely 2C8, 2C9, 2C18 and 2C19. CYP2Cs are the second most abundant CYP proteins in human liver and the CYP2C subfamily consists of three members, comprising about 20 % of the total P450 enzymes. In humans, CYP2C9 is the main CYP2C, followed by CYP2C8 and CYP2C19, while CYP2C18 is not expressed in liver (Pelkonen et al., 2008; Edwards et al., 1998; Shimada et al., 1994; Gray et al., 1995; Richardson et al., 1997). CYP2C9 is a major CYP2C isoform in the human liver, and it is one of several CYP2C genes clustered in a 500kb region on the proximal 10q24 chromosomal region (Gray et al., 1995; Goldstein and de Morais, 1994). In Caucasian populations, the frequencies of the two variant alleles, CYP2C9*2 and CYP2C9*3, range from 7% to 19% (Furuya et al., 1995; Ingelman-Sundberg et al., 1999; Miners & Birkett, 1998; Stubbins et al., 1996; Sullivan-Klose et al., 1996; Yasar et al., 1999). CYP2C19, another member of the CYP2C enzyme family, represents approximately 5% of the total hepatic CYPs and metabolizes drugs that are amides or weak bases with two hydrogen bond acceptors (Pelkonen et al., 2008; Lewis, 2004; Musana & Wilke, 2005). Poor metabolizers with low CYP2C19 activity represent 3 to 5% of Caucasians and African-Americans, and 12 to 23% of most Asian populations (Goldstein, 2001).

2.5 CYP2D subfamily

CYP2D6 represents 1 to 5% of the total CYP, and approximately 3.5 and 5-10% of the Caucasian population are ultra-rapid and poor metabolizer's for this enzyme, respectively. The CYP2D6 gene is clearly the most polymorphic of all known cytochrome P450s; more than 75 polymorphisms have been identified. Four alleles account for > 95% of the functional variation observed in the general population (Pelkonen et al., 2008; Shimada et al., 1994; Musana & Wilke, 2005; Al, Omari, A., & Murry, 2007; Ingelman-Sundberg, 2004; Zanger et al., 2004).

2.6 CYP2E subfamily

Only one gene belonging to this subfamily, namely CYP2E1, has been identified (Nelson et al., 1996; Nelson et al., 2004). CYP2E1 is one of the most abundant hepatic CYPs, represents 15% of the total P450 and it is also expressed in lung and brain (Pelkonen et al., 2008; Raunio et al., 1995).

2.7 CYP3A subfamily

In humans, the CYP3A subfamily contains three functional proteins, CYP3A4, CYP3A5, and CYP3A7, and one pseudoprotein, CYP3A34. The human CYP3 family constitutes approximately 30 % of total hepatic P450 and is estimated to mediate the metabolism of

around 50% of prescribed drugs as well as a variety of environmental chemicals and other xenobiotics. Because of the large number of drugs metabolized by CYP3A4, it frequently plays a role in a number of drug-drug interactions (Pelkonen et al., 2008; Shimada et al., 1994; Musana & Wilke, 2005; Bertz & Granneman, 1997; Domanski et al., 2001; Imaoka et al., 1996; Rostami-Hodjegan & Tucker, 2007).

CYP3A4 is the major form of P450 expressed in human liver. It is also the major P450 expressed in human gastrointestinal tract, and intestinal metabolism of CYP3A4 substrate can contribute significantly to first-pass elimination of orally ingested xenobiotics (Guengerich, 1995; Guengerich, 1999). X-ray crystallography studies demonstrated that CYP3A4 has a very large and flexible active site, allowing it to oxidize either large substrates such as erythromycin and cyclosporine or multiple smaller ligands (Scott & Halpert, 2005; Tang & Stearns, 2001).

CYP3A5 is a minor polymorphic CYP isoform in human liver in addition to the intestine (Lin et al., 2002; Paine et al., 1997) and kidney (Haehner et al., 1996). Functional CYP3A5 is expressed in approximately 20% of Caucasians and about 67% of African-Americans (Kuehl et al., 2001). CYP3A5 may have a significant role in drug metabolism particularly in populations expressing high levels of CYP3A5 and/or on co-medications known to inhibit CYP3A4 (Soars et al., 2006).

Expression of CYP3A7 protein is mainly confined to fetal and newborn livers, although in rare cases CYP3A7 mRNA has been detected in adults (Hakkola et al., 2001; Kitada & Kamataki, 1994; Schuetz et al., 1994).

3. Xenobiotic biotransformation

Xenobiotic biotransformation is the process by which lipophilic foreign compounds are metabolized through enzymatic catalysis to hydrophilic metabolites that are eliminated directly or after conjugation with endogenous cofactors via renal or biliary excretion. These metabolic enzymes are divided into two groups, Phase I and Phase II enzymes (Rendic & Di Carlo, 1997; Oesch et al., 2000).

Phase I products are not usually eliminated rapidly, but undergo a subsequent reaction in which an endogenous substrate such as glucuronic acid, sulfuric acid, acetic acid, or an amino acid combines with the newly established functional group to form a highly polar conjugate to make them more easily excreted. Sulfation, glucuronidation and glutathione conjugation are the most prevalent classes of phase II metabolism, which may occur directly on the parent compounds that contain appropriate structural motifs, or, as is usually the case, on functional groups added or exposed by Phase I oxidation (LeBlanc & Dauterman, 2001; Rose & Hodgson, 2004; Zamek-Gliszcynski et al., 2006).

3.1 In vitro and human-derived techniques for testing xenobiotic metabolism

In order to study the metabolism and interactions of pesticides in humans we have to rely upon *in vitro* and human-derived techniques. *In vitro* systems have become an integral part of drug metabolism studies as well as throughout the drug discovery process and in academic research (Pelkonen et al., 2005; Pelkonen & Raunio, 2005; Lin & Lu, 1997). *In vitro* approaches to predict human clearance have become more frequent with the increase in the availability of human-derived materials (Skett et al., 1995). All models have certain advantages and disadvantages, but the common advantage to these approaches is the reduction of the complexity of the study system. However, the use of *in vitro* models is always a compromise between convince and relevance (Pelkonen et al., 2005; Brandon et al.,

2003; Pelkonen & Turpeinen, 2007; Pelkonen & Turpeinen, 2007). An overview of different *in vitro* models and their advantages and disadvantages are collected in Table 1.

Enzyme sources	Availability	Advantages	Disadvantages
Liver homogenates ^a	Relatively good. Commercially available. Human liver samples obtained under proper ethical permission.	Contains basically all hepatic enzymes.	Liver architecture lost. Cofactors are necessary.
Microsomes ^a	Relatively good, from transplantations or commercial sources.	Contains important rate-limiting enzymes. Inexpensive technique. Easy storage. Study of individual, gender-, and species-specific biotransformation.	Contains only CYP and UGTs. Requires strictly specific substrates and inhibitors or antibodies. Cofactor addition necessary.
cDNA-expressed individual CYPs ^b	Commercially available	The role of individual CYPs in the metabolism can be easily studied. Different genotypes. High enzyme activities.	The effect of only one enzyme at a time can be evaluated. Problems in extrapolation to HLM and <i>in vivo</i> .
Primary hepatocytes ^{c,d}	Difficult to obtain, relatively healthy tissue needed. Commercially available	Contains the whole complement of CYPs cellularly integrated. The induction effect can be studied. Well established and characterized. Transporters still present and operational.	Requires specific techniques and well established procedures. The levels of many CYPs decrease rapidly during cultivation. Cell damage during isolation.
Liver slices ^e	Difficult to obtain, fresh tissue needed.	Contains the whole complement of CYPs and cell-cell connections. The induction, morphology and interindividual variation can be studied.	Requires specific techniques and well established procedures
Immortalized cell lines ^f	Available upon request, not many characterized cell lines exist.	Non-limited source of enzymes. Easy to culture. Relatively stable enzyme expression level. The induction effect can be studied.	The expression of most CYPs is poor.

^a (Kremers, 1999); ^b (Rodrigues, 1999); ^c (Guillouzo, 1995); ^d (Gomez-Lechon et al., 2004); ^e (Olinga et al., 1998); ^f (Allen et al., 2005).

Table 1. An overview of different *in vitro* models and their advantages and disadvantages (modified from (Pelkonen et al., 2005; Pelkonen & Raunio, 2005; Brandon et al., 2003; Pelkonen & Turpeinen, 2007)).

3.2 In vitro characterization of the metabolism and metabolic interactions of xenobiotics

The aim of *in vitro* characterization is to produce relevant and useful information on metabolism and interactions to anticipate, and even to predict, what happens in man. Each *in vitro* model has its own set of advantages and disadvantages as they range from simple to more complex systems: individual enzymes, subcellular fractions, cellular systems, liver slices and whole organ, respectively (Pelkonen et al., 2005; Pelkonen & Raunio, 2005; Brandon et al., 2003). To understand some of the factors related to xenobiotic metabolism that can influence the achievement of these aims, there are several important points to consider such as (Pelkonen et al., 2005; Pelkonen & Raunio, 2005; Pelkonen & Turpeinen, 2007; Hodgson & Rose, 2005):

- Determination of the metabolic stability of the compound
- Identification of reactive metabolites
- Evaluation of the variation between species
- Identification of human CYPs and their isoforms involved in the activation or detoxification
- Evaluation of the variation between individuals
- Identification of individuals and subpopulations at increased risk
- Overall improvement of the process of human risk assessment

An overview of different *in vitro* studies for the characterization of metabolism and metabolic interactions of xenobiotics are collected in Table 2.

<i>In vitro</i> test	<i>In vitro</i> model	Parameters	Extrapolations
Metabolic stability	Microsomes	Disappearance of the parent molecule or appearance of (main) metabolites	Intrinsic clearance
	Homogenates		Interindividual variability
	Cells		Interspecies differences
	Slices		
Metabolite identification and quantitation	Microsomes	Tentative identification by (e.g.) LC/TOF-MS	Metabolic routes
	Homogenates		Semi-quantitative
	Cells		Interspecies differences
	Slices		
Identification of metabolizing enzymes	Microsomes with inhibitors or inhibitory antibodies	Relative ability of enzymes to metabolize a compound	Prediction of effects of genetic, environmental and pathological factors
	Recombinant CYPs		
	Hepatocytes		Interindividual variability
Enzyme inhibition	Microsomes	Inhibition of specific model substrate	Potential interactions
	Recombinant enzymes		
	Hepatocytes		
Enzyme induction	Cells	Induction of CYP activities or mRNA	Induction potential of a substance
	Slices		
	Permanent cell lines		

Table 2. *In vitro* studies for the characterization of the metabolism and metabolic interactions of xenobiotics (modified from (Pelkonen et al., 2005; Pelkonen and Raunio, 2005)).

4. The contribution of CYPs to the metabolism of xenobiotics in human

4.1 CYP1A subfamily

The catalytic activities of CYP1A2 have been reviewed by Pelkonen *et al.* (Pelkonen et al., 2008). CYP1A2 has a major role in the metabolism of many important chemicals such as caffeine (Butler *et al.*, 1989; Tassaneeyakul *et al.*, 1992), phenacetin (Sesardic *et al.*, 1990; Venkatakrishnan *et al.*, 1998), theophylline (Sarkar & Jackson, 1994; Tjia *et al.*, 1996), clozapine (Fang *et al.*, 1998), melatonin (Faccioli *et al.*, 2001; von Bahr *et al.*, 2000), and tizanidine (Granfors *et al.*, 2004a; Granfors *et al.*, 2004b). CYP1A1 is a major enzyme in the metabolism of a number of insecticides and herbicides (Lang *et al.*, 1997; Tang *et al.*, 2002; Abass *et al.*, 2010; Abass *et al.*, 2007c). CYP1A2 mediates herbicides (Lang *et al.*, 1997; Abass *et al.*, 2007c; Nagahori *et al.*, 2000), insecticides (Tang *et al.*, 2002; Stresser & Kupfer, 1998; Foxenberg *et al.*, 2007; Mutch & Williams, 2006), and pyrethroids metabolism (Scollon *et al.*, 2009).

4.2 CYP2A subfamily

It has been shown that CYP2A6 has a major role in the metabolism of nicotine *in vitro* and *in vivo* (Kitagawa *et al.*, 1999; Messina *et al.*, 1997; Nakajima *et al.*, 1996a; Nakajima *et al.*, 1996b; Yamazaki *et al.*, 1999) and in the activation of aflatoxin B1 (Yun *et al.*, 1991; Salomäe *et al.*, 1993). More substrates and inhibitors currently known to be metabolized by or to interact with CYP2A6 *in vitro* and *in vivo* have been summarized by Pelkonen and co-workers (Pelkonen *et al.*, 2008; Pelkonen *et al.*, 2000). CYP2A6 participates in the metabolism of quite a few pesticides such as carbaryl, imidacloprid, DEET, carbosulfan and diuron (Tang *et al.*, 2002; Abass *et al.*, 2010; Abass *et al.*, 2007c; Schulz-Jander and Casida, 2002; Usmani *et al.*, 2002).

4.3 CYP2B subfamily

CYP2B6 is known to metabolize a large number of substrates including drugs, pesticides and environmental chemicals, many of which have been described in detail in reviews (see e.g. (Ekins & Wrighton, 1999; Hodgson & Rose, 2007b; Turpeinen *et al.*, 2006)). Several clinically used drugs such as cyclophosphamide, bupropion, S-mephenytoin, diazepam, ifosamide and efavirenz are metabolized in part by CYP2B6 (Granvil *et al.*, 1999; Haas *et al.*, 2004; Huang *et al.*, 2000; Jinno *et al.*, 2003; Roy *et al.*, 1999b; Roy *et al.*, 1999a). CYP2B6 appears to activate and detoxify a number of precarcinogens (Code *et al.*, 1997; Smith *et al.*, 2003). CYP2B6 plays a major role in pesticides metabolism. CYP2B6 mediates herbicides N-dealkoxylation (Coleman *et al.*, 2000); organophosphate insecticides desulfuration (Foxenberg *et al.*, 2007; Mutch & Williams, 2006; Buratti *et al.*, 2005; Leoni *et al.*, 2008; Sams *et al.*, 2000; Tang *et al.*, 2001); organochlorine and carbamate insecticides sulfoxidation (Abass *et al.*, 2010; Casabar *et al.*, 2006; Lee *et al.*, 2006); fungicide metalaxyl O-demethylation and lactone formation (Abass *et al.*, 2007b).

4.4 CYP2C subfamily

CYP2C8 mediates amodiaquine N-deethylation, which is the selective marker activity, paclitaxel 6 α -hydroxylation and cerivastatin demethylation (Li *et al.*, 2002; Rahman *et al.*, 1994). A few insecticides are mainly metabolized by CYP2C8 such as parathion, deltamethrin, esfenvalerate, and β -cyfluthrin (Mutch & Williams, 2006; Scollon *et al.*, 2009; Mutch *et al.*, 2003; Godin *et al.*, 2007).

CYP2C9 is responsible for the metabolism of the S-isomer of warfarin (Rettie *et al.*, 1992). CYP2C9 also metabolizes tolbutamide, the selective marker, glipizide, fluvastatin,

phenytoin, several non-steroidal anti-inflammatory agents and many other drug groups (Miners & Birkett, 1998; Doecke et al., 1991; Kirchheimer & Brockmoller, 2005; Rettie and Jones, 2005). CYP2C9 is found to be involved in the metabolism of pesticides such as pyrethroid insecticides (Scollon et al., 2009; Godin et al., 2007), as well as organophosphorus insecticides (Leoni et al., 2008; Usmani et al., 2004).

CYP2C19 participates in the metabolism of many commonly used drugs including the antiepileptics phenytoin and mephenytoin (Bajpai et al., 1996; Komatsu et al., 2000; Tsao et al., 2001; Wrighton et al., 1993)(Bajpai et al. 1996, Komatsu et al. 2000, Tsao et al. 2001, Wrighton et al. 1993), selective serotonin receptor inhibitors citalopram and sertraline (Kobayashi et al., 1997; von Moltke et al., 2001), the psychoactive drugs amitriptyline (Venkatakrishnan et al., 1998; Jiang et al., 2002) and diazepam, among others (Jung et al., 1997). Among the substrates of CYP2C19 are several widely used pesticides such as the phosphorothioate insecticides (Foxenberg et al., 2007; Mutch & Williams, 2006; Leoni et al., 2008; Tang et al., 2001; Usmani et al., 2004; Buratti et al., 2002; Kappers et al., 2001), as well as the pyrethroid insecticides (Scollon et al., 2009; Godin et al., 2007).

4.5 CYP2D subfamily

CYP2D6 metabolizes approximately 20 % of all commonly prescribed drugs *in vivo* (Brockmöller et al. 2000). For example, CYP2D6 contributes to the metabolism of betablockers metoprolol and timolol (Johnson & Burlew 1996, Volotinen et al. 2007) and the psychotropic agents amitriptyline and haloperidol (Coutts et al. 1997, Fang et al. 1997, Fang et al. 2001, Halling et al. 2008, Someya et al. 2003). Dextromethrophan O-demethylation is the most used *in vitro* model reaction for CYP2D6 activity (Kronbach et al. 1987, Park et al. 1984). Known pesticide substrates for CYP2D6 include phosphorothioate insecticides (Mutch et al. 2003, Mutch & Williams 2006, Sams et al. 2000, Usmani et al. 2004b) as well as (Johnson and Burlew, 1996; Volotinen et al., 2007) carbamate insecticide (Tang et al., 2002). CYP2D6 is also involved in the N-dealkylation of the atrazine and diuron herbicides (Lang et al., 1997; Abass et al., 2007c).

4.6 CYP2E subfamily

The metabolism of very few clinically important drugs such as paracetamol, caffeine, acetaminophen, enflurane and halothane seems to be mediated to some extent by CYP2E1 (Gu et al., 1992; Lee et al., 1996; Raucy et al., 1993; Thummel et al., 1993). Chlorzoxazone is probably the most used *in vitro* model substrate for CYP2E1 activity (Peter et al., 1990). Few pesticides have been reported to be metabolized at least in part by human CYP2E1 such as atrazine, carbaryl, parathion, imidacloprid and diuron (Lang et al., 1997; Tang et al., 2002; Abass et al., 2007c; Mutch & Williams, 2006; Schulz-Jander & Casida, 2002; Mutch et al., 2003).

4.7 CYP3A subfamily

CYP3A4 participates in the metabolism of several clinically important drugs such as triazolam, simvastatin, atorvastatin, and quinidine (Rendic & Di Carlo, 1997; Bertz & Granneman, 1997). Detailed characteristics of several CYP3A4 substrates and inhibitors were summarized recently by Liu et al. (Liu et al., 2007). The known pesticides mainly metabolized by CYP3A4 belong to several chemical groups such as, carbamate, phosphorothioate, chlorinated cyclodiene and neonicotinoid insecticides (Tang et al., 2002; Abass et al., 2010; Mutch & Williams, 2006; Schulz-Jander & Casida, 2002; Buratti et al., 2005;

Sams et al., 2000; Tang et al., 2001; Casabar et al., 2006; Lee et al., 2006; Mutch et al., 2003; Usmani et al., 2004; Buratti et al., 2002; Buratti et al., 2003; Buratti & Testai, 2007; Butler and Murray, 1997), herbicides (Abass et al., 2007c; Coleman et al., 2000), fungicides (Abass et al., 2007b; Abass et al., 2009; Mazur et al., 2007), and organotin biocide (Ohhira et al., 2006).

CYP3A5 mediates midazolam, alprazolam and mifepristone metabolism (Christopher Gorski et al., 1994; Galetin et al., 2004; Hirota et al., 2001; Huang et al., 2004; Khan et al., 2002; Williams et al., 2002). Alprazolam has been suggested as a selective probe for CYP3A5 (Galetin et al., 2004). The metabolism of a number of organophosphate and pyrethroid insecticides has been reported to be mediated by CYP3A5 (Mutch & Williams, 2006; Mutch et al., 2003; Godin et al., 2007).

CYP3A7 has similar catalytic properties compared with other CYP3A enzymes, including testosterone 6 β -hydroxylation (Kitada et al., 1985; Kitada et al., 1987; Kitada et al., 1991).

5. The impact of modern analytical techniques in xenobiotics metabolism

5.1 Mass spectrometric methods in metabolism studies.

Traditionally metabolism studies were performed using gas chromatography-mass spectrometry (GC-MS). Because metabolites are usually polar molecules with high molecular masses, they have to be derivatised before measurement. After derivatisation the measured analyte is not anymore original metabolite but a less polar compound which is possible to vaporise and use in GC. Derivatisation can cause errors to the measurements and it is usually the most time and labour demanding phase which causes extra costs (Sheehan, 2002). In metabolism studies biggest problem of GC-MS is its lower sensitivity compared with modern mass spectrometric methods. Nevertheless GC-MS is still a useful method also in metabolism studies especially with thermally stable volatile compounds.

Nowadays the primary method used in metabolism studies is liquid chromatogram-mass spectrometry (LC-MS). Liquid chromatography is an old technique to separate polar compounds in liquid phase. However, it took quite a long time to develop a reliable technique to connect LC to the mass spectrometer, because the eluent solvent has to be vaporized before actual MS measurements which are performed in high vacuum. However after the introduction of electrospray ionisation (ESI) development has been very rapid during last 20 years and the performance of instruments has steadily improved. Usually ESI is the best choice for the ionization of polar metabolites but there are also other common ionisation methods like APCI (atmospheric pressure chemical ionisation) and APPI (atmospheric pressure photo-ionisation), which can be used to ionise less polar compounds. ESI can be run either in positive or negative modes and the best choice is dependent on the specific analyte. During ionisation hydrogen is either combined with the analyte to produce [M+H]⁺ ion or broken away to produce [M-H]⁻ ion, which can be accelerated within electric field. In the same time usually also other adducts, like sodium and potassium adducts, are formed. Sometimes other adducts can cause problems or decrease the sensitivity of the method. In addition other compounds that elute at same time from LC flow can reduce or block totally the ionisation of the analyte and cause errors to the measurements. This phenomenon is called ion suppression and it is quite common in ESI (Jessome & Volmer, 2006).

The most useful sample handling procedure to be used in metabolism studies with LC-MS is protein precipitation. It is performed easily by addition of organic solvent, either methanol or acetonitrile, to the samples. Samples are mixed and centrifuged to get clear supernatant. Usually after protein precipitation samples are clean enough to be analyzed directly, but

also other sample handling method may be needed with samples containing a lot of lipids or salts (Rossi, 2002). Different extraction methods, like SPE (solid phase extraction) or liquid-liquid extraction are then a better choice. However they are more expensive and time consuming methods.

Already HPLC (high performance liquid chromatography) is able to separate metabolites directly without any modifications. Compared to the GC, the resolution of HPLC is quite poor. Because mass spectrometric methods can measure compounds coming to the instrument at the same time, this has not been so big a problem. During the last five years liquid chromatography has improved considerably after introduction of ultra performance liquid chromatography (UPLC). UPLC instruments can work in higher operation pressures (up to 15.000 psi) which makes possible to use smaller particles and diameters in columns and to improve resolution, speed and sensitivity of the method. A typical run in UPLC can be just 5 minutes to analyse several different compounds.

Mass spectrometry is a superior method in the metabolism studies because of its high sensitivity. Although mass spectrometry is usually understood as one concept, it actually consist of several different types of instruments and techniques. Different types of instruments have specific advantages and consequently each individual type suits best for certain kind(s) of measurements. In the identification of metabolites time of flight mass spectrometry (TOF) is the best option. It can detect all ionized compounds simultaneously which improve the sensitivity compared to scanning instruments (Fountain, 2002). With help of the TOF instruments accurate mass of the analyte (± 5 ppm) can be measured and elemental composition can be calculated. Modern instruments can easily reach 1 ppm mass accuracy and use isotope patterns of analytes to solve the right elemental composition with few potential possibilities. This kind of identification can be used to find different metabolites in samples, because masses of potential metabolites can usually be calculated before measurements. There are also softwares, such as Metabolynx (Waters Corp., Milford, MA, USA), which can search potential metabolites automatically from mass chromatograms and help a lot in data processing.

Additional structural information can be achieved with help of Q-TOF or triple quadrupole instruments. Measured analytes can be decomposed by collision with gas molecules (CID, collision induced dissociation) to produce fragment ions. In Q-TOF instruments accurate mass of fragment ions can be also measured to resolve molecular masses of fragments. In most cases fragmentation produces information about location of possible biotransformations. Because fragmentation is compound-specific, fragments can be used for identification purposes if they are known from previous measurements. However fragmentation is not as universal as in EI-ionisation (electron ionisation) of GC-MS instruments because it is partly instrument specific. With ion trap instruments even produced fragment ion can be selected and collided again to produce new smaller fragment ions. To resolve the structure of a metabolite completely other methods like nuclear magnetic resonance (NMR) or x-ray crystallography are usually required.

Knowledge about fragmentation of the analyte is useful also in quantitative measurements. Quantifications are usually performed in triple quadrupole instruments, where fragmentation can be used to increase selectivity of the measurements. The mode of the measurement is called multiple reaction monitoring (MRM), because several compounds can be measured simultaneously. In triple quadrupole instruments the first quadrupole selects the measured analyte, the second one decomposes it and the third passes the formed fragment to the detector. Because fragmentation is specific to every analyte, only right one is

measured even if compounds with the same molecular mass come to the instrument at the same time. This kind of high selectivity makes also possible to measure several compounds at the same time even when they are not separated in liquid chromatography. In triple quadrupole instruments dynamic range is usually at least 5 orders of magnitude what is enough for quantification purposes. Earlier TOF instruments were saturated at quite low concentrations and could not be used for quantification purposes. Modern TOF instruments however can be used for quantification at least to a certain extent.

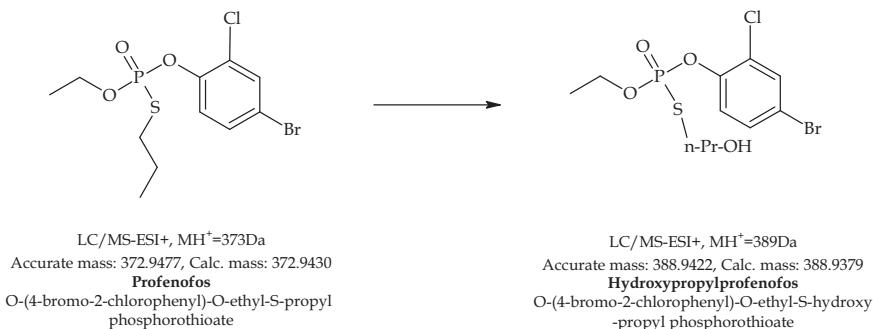
Newest technological addition to mass spectrometry is ion mobility. Ion mobility is a small gas filled drift tube in instruments, which ions travel through within electric field. Drift tube will separate compounds based on their shape and size in addition to mass and charge as in conventional instruments. Ion mobility is quite an old technique but just recently it has been combined with commercial mass spectrometers like Synapt HDMS (Waters Corp., Milford, MA, USA) (Kanu et al., 2008). Ion mobility can be used to clean important analytes from sample matrix and to separate very similar compounds like isomers from each other. Because technique is so new, its real practical significance in metabolism studies is still unclear.

Figure 3 presents a practical example about mass spectrometric measurements of the pesticide profenofos and its metabolite hydroxypropylprofenofos (Abass et al., 2007a). Accurate mass measurements were performed by Micromass LCT-TOF (Micromass, Altrincham, UK) using leucine enkephalin ($[M+H]^+$ at m/z 556.2771) as a lock mass compound. Error in accurate mass measurements of hydroxypropylprofenofos was 4.3 mDa. Fragmentations of hydroxypropylprofenofos were determined by Micromass Quattro II triple quadrupole instruments. In the first fragmentation hydroxypropylprofenofos loses ethanol to produce fragment of m/z = 343 Da. In the second step propanol is released to produce fragment of 285 Da. Difference in molecular masses of these two fragments indicates that hydroxylation has to be located in S-propyl moiety of the metabolite. Finally quantifications were performed in multiple reaction monitoring mode (MRM) of triple quadrupole instruments. Quadrupole 1 passes only hydroxypropylprofenofos (molecular mass 389 Da) or compounds with the same molecular mass. After quadrupole 1 hydroxypropylprofenofos will fragment in collision cell with help of argon gas and collision energy (CE= 20 eV) to produce a specific fragment of m/z 343 Da. In the final step only fragment 343 will pass quadrupole 3 and its amount is determined in the detector of the instrument. After calibration of the instrument with reference standards, the real amount of hydroxypropylprofenofos can be determined.

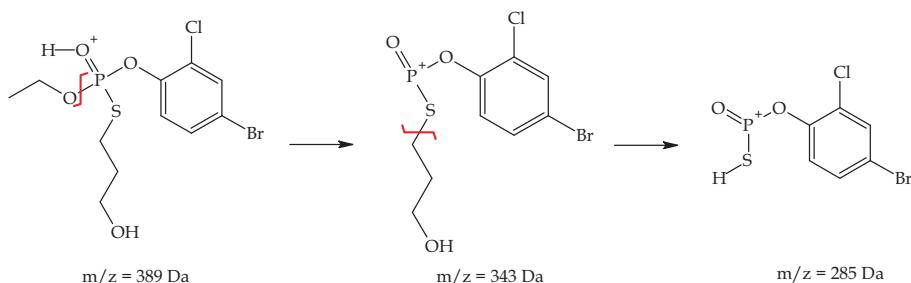
5.2 Nuclear Magnetic Resonance spectrometry in the metabolism studies.

Nuclear Magnetic Resonance spectroscopy (NMR) is a powerful analytical tool in studies of solid, gaseous and liquid samples. The versatility of the technique and the long relaxation times of the nuclear spins allow for probing various different properties of the samples. An even normal, simple one-dimensional spectrum contains valuable information about the sample concentration, electron distributions of the molecule, spatial proximities of different chemical sites and electrostatic connectivities between different nuclei of the molecule. The full potential of NMR can be unleashed by going into higher dimensional NMR spectroscopy. In typical two-, or three-dimensional NMR spectra one can probe spatial proximities of various nuclei, do diffusion separated spectroscopy, probe for heteronuclear connectivities multiple bonds away, or characterize intermolecular dipolar interactions at the protein-ligand complex interface.

Accurate mass measurement of hydroxypropylprofenofos by time-of-flight mass spectrometer:



The fragmentation of hydroxypropylprofenofos by triple quadrupole mass spectrometer:



The quantification of hydroxypropylprofenofos by triple quadrupole mass spectrometer:

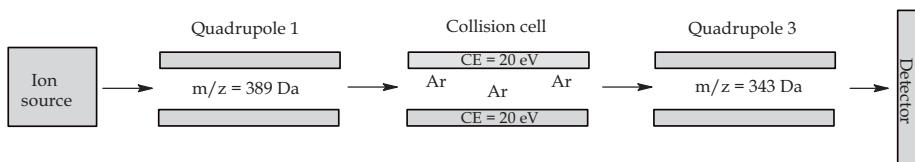


Fig. 3. Mass spectrometric measurements, accurate mass, fragmentations and quantifications, performed to study hydroxylation of profenofos in human hepatic subcellular fractions.

The biggest drawback of the NMR spectroscopy is its inherently low sensitivity, because the observed signal arises from the population difference of spin states. This population difference follows Boltzmann distribution and is quite low even at reasonably high magnetic fields used at modern NMR spectrometers. As the experiments are performed close to room or physiological temperature, there is only population difference of about 10 spins for every million spins in the sample.

Two recent sensitivity enhancing methods are cryogenic cooling of the probe-head electronics and miniaturization of the sample size. Equipment for both of these have been

commercially available now for several years and when combined the resulting cryo-microprobe would give up to 15 fold increase in sensitivity compared to regular room temperature probe head (Kovacs et al., 2005). Several articles have recently been published where full NMR analyses of complex natural products have been made using only nanomoles of material (would be equal to 1 µg if molecular mass is 1000) (Dalisay & Molinski, 2009; Choi et al., 2010a; Choi et al., 2010b; Djukovic et al., 2008).

If one wishes the acquisition parameters of NMR experiment can be set to provide quantitative spectrum. Typically the delay between individual transitions needs to be lengthened to allow full relaxation of spins before next transition. In simplest form the integral values of the individual resonances in spectrum give information of how many equivalent spins are present. Whenever there are modifications in chemical structure the change in integral provides valuable information on the chemical site of the modification (Holzgrabe, 2010).

The Chemical shift is a sensitive measure of the electronic surrounding of individual nuclei of a molecule. Even smallest changes in the chemical structure can cause peaks to resonate at slightly different frequency at the chemical shift range. Addition of the electronegative substituents to the molecule changes the chemical shifts of the resonances of the nearby nuclei. In favorable case the change in chemical shift can be observed for several nucleus many bonds away from the origin of modification site.

The signal fine structure, the splittings caused by spin-spin couplings, provides additional sensitive measure of the topology of the nucleus in the molecule. Change in number of nearby nucleus or even just a conformational change can be detected as a change observed coupling pattern caused by the spin-spin coupling.

In metabolic studies NMR spectroscopy is best utilized when used as a complementary technique to the mass spectrometric techniques. For instance the position isomerism studies are often quite tricky or even impossible to solve by mass spectroscopy e.g. what is the substitution pattern of the aromatic ring or which carbon of the aliphatic chain was hydroxylated. These questions can occasionally be answered in minutes by single ¹H NMR spectrum. Of course more challenging structural questions take longer and might require acquisition of several multi-dimensional data sets.

For the illustration of the position isomerism detection powers of NMR spectroscopy several simulated NMR spectra of the profenofos and the hydroxypropylprofenofos are displayed in figure 4. On the left are the aromatic signals of the profenofos and the corresponding spectrum if the bromine was in ortho position to the chlorine. The difference in signal positions and splitting patterns is clear. On the right are spectra of the propyl moiety of the profenofos and the hydroxypropyl moiety of the hydroxypropylprofenofos where the hydroxylation has occurred on terminal carbon 3 or in carbon 2.

6. Conclusion

The cytochrome P450 (CYP) superfamily comprises a broad class of phase I oxidative enzymes that catalyze many hepatic metabolic processes. Human CYPs have broad substrate specificity and enzymes in families 1-3 function mostly in the metabolism of a wide variety of xenobiotics. In human liver, CYP3A4 is found in the highest abundance and it metabolizes the greatest number of drugs and a very large number of other xenobiotics. CYP enzymes in humans as well as in other species have been intensively studied during recent years. It is now possible to characterize metabolism, metabolic interactions and to

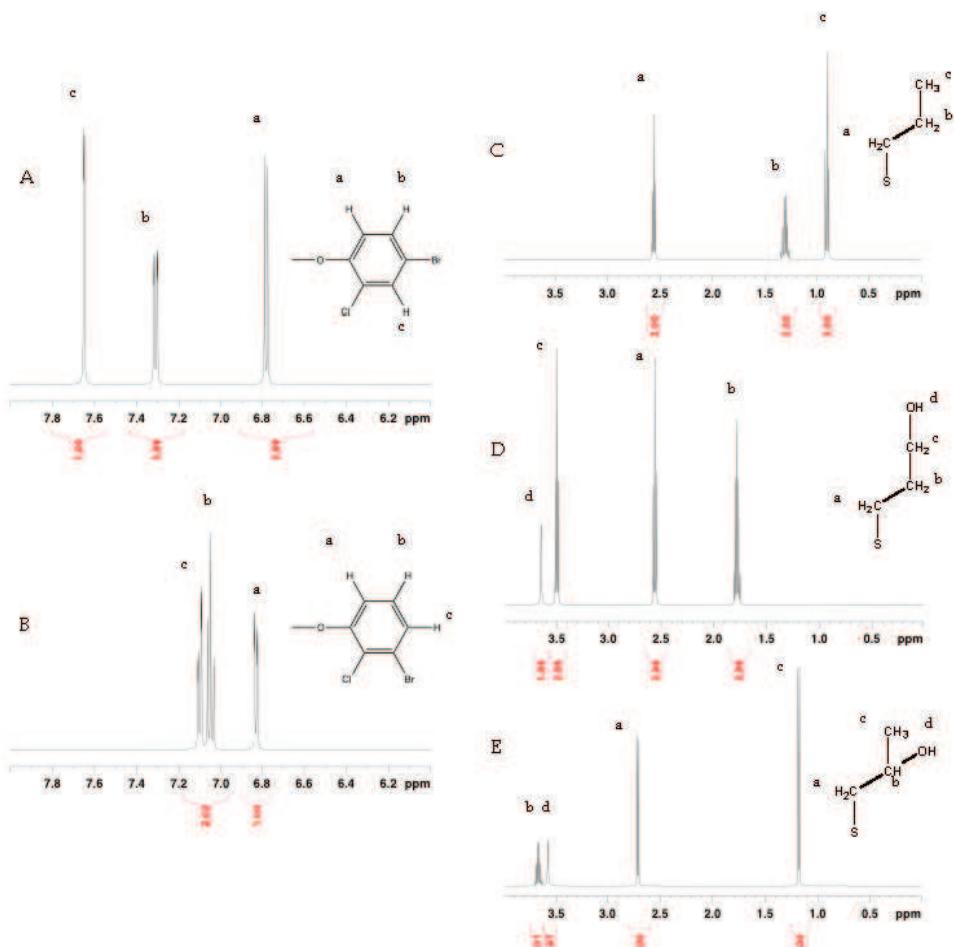


Fig. 4. An example of the effect of small changes in molecular structure to the outlook of ¹H NMR spectrum illustrated by 5 simulated NMR spectra. The values used for chemical shifts and coupling constants are only approximate and are presented for illustration purposes only. The values of signal integration are displayed below the frequency scale.

determine which P450 is involved in the metabolism of a certain xenobiotic by different *in vitro* approaches. The toxicity of many types of pesticides is mediated by enzymatic biotransformation reactions in the body. Recently, a number of papers have been published on the activity of human P450s involved in the metabolism of pesticides and these activities may result in activation and/or detoxification reactions.

The aim of *in vitro* characterization is to produce relevant and useful information on metabolism and interactions to predict what happens *in vivo* in human. To understand some of the factors related to xenobiotics, including pesticides, metabolism that can influence the achievement of these aims, there are several important points to consider such as metabolic stability, metabolic routes and fractional proportions, metabolizing enzymes

and potential interactions. In this review we described the human xenobiotic-metabolizing enzymes CYPs system; briefly illustrate in vitro human-derived techniques for studying xenobiotic metabolism and in vitro characterization of metabolic characteristics; review the role of CYPs in the metabolism of xenobiotics, including drugs and pesticides, in human in vitro; and finally describe the impact of modern analytical techniques in xenobiotics metabolism.

7. References

- Abass, K.; Reponen, P. & Pelkonen, O. (2009). Metabolic and interactions properties of selected fungicides, In: *Fungicides: chemistry, environmental impact and health effects*, De Costa, P. & Bezerra, P., (Ed.), (25-62), Nova Science Publisher, ISBN: 978-1-60692-631-4, New York
- Abass, K.; Reponen, P.; Mattila, S. & Pelkonen, O. (2010). Metabolism of carbosulfan II. Human interindividual variability in its in vitro hepatic biotransformation and the identification of the cytochrome P450 isoforms involved. *Chem. Biol. Interact.*, Vol. 185, No. 3, (163-173).
- Abass, K.; Reponen, P.; Jalonens, J. & Pelkonen, O. (2007a). In vitro metabolism and interaction of profenofos by human, mouse and rat liver preparations. *Pestic. Biochem. Physiol.*, Vol. 87, No. 3, (238-247).
- Abass, K.; Reponen, P.; Jalonens, J. & Pelkonen, O. (2007b). In vitro metabolism and interactions of the fungicide metalaxyl in human liver preparations. *Environ. Toxicol. Pharmacol.*, Vol. 23, No. 1, (39-47).
- Abass, K.; Reponen, P.; Turpeinen, M.; Jalonens, J. & Pelkonen, O. (2007c). Characterization of diuron N-demethylation by mammalian hepatic microsomes and cDNA-expressed human cytochrome P450 enzymes. *Drug Metab. Dispos.*, Vol. 35, No. 9, (1634-1641).
- Adams, D.R.; Jones, A.M.; Plopper, C.G.; Serabjit-Singh, C.J. & Philpot, R.M. (1991). Distribution of cytochrome P-450 monooxygenase enzymes in the nasal mucosa of hamster and rat. *Am. J. Anat.*, Vol. 190, No. 3, (291-298).
- Al, Omari, A., & Murry, D., J. (2007). Pharmacogenetics of the cytochrome P450 enzyme system: review of current knowledge and clinical significance. *J. Pharm. Pract.*, Vol. 20, No. 3, (206-218).
- Alavanja, M.C.R.; Hoppin, J.A. & Kamel, F. (2004). Health effects of chronic pesticide exposure: cancer and neurotoxicity. *Annu. Rev. Public Health*, Vol. 25, No. 1, (155-197).
- Allen, D.D.; Caviedes, R.; Cárdenas, A.M.; Shimahara, T.; Segura-Aguilar, J. & Caviedes, P.A. (2005). Cell lines as in vitro models for drug screening and toxicity studies. *Drug Dev. Ind. Pharm.*, Vol. 31, No. 8, (757).
- Bajpai, M.; Roskos, L.; Shen, D. & Levy, R. (1996). Roles of cytochrome P4502C9 and cytochrome P4502C19 in the stereoselective metabolism of phenytoin to its major metabolite. *Drug Metab. Dispos.*, Vol. 24, No. 12, (1401-1403).
- Bergh, A.F. & Strobel, H.W. (1992). Reconstitution of the brain mixed function oxidase system: Purification of NADPH-cytochrome P450 reductase and partial purification of cytochrome P450 from whole rat brain. *J. Neurochem.*, Vol. 59, No. 2, (575-581).

- Bertz, R.J. & Granneman, G.R. (1997). Use of in Vitro and in vivo data to estimate the likelihood of metabolic pharmacokinetic interactions. *Clin. Pharmacokinet.*, Vol. 32, No. 3, (210-258).
- Boobis, A.R.; Ossendorp, B.C.; Banasiak, U.; Hamey, P.Y.; Sebestyen, I. & Moretto, A. (2008). Cumulative risk assessment of pesticide residues in food. *Toxicol. Lett.*, Vol. 180, No. 2, (137-150).
- Brandon, E.F.A.; Raap, C.D.; Meijerman, I.; Beijnen, J.H. & Schellens, J.H.M. (2003). An update on in vitro test methods in human hepatic drug biotransformation research: pros and cons. *Toxicol. Appl. Pharmacol.*, Vol. 189, No. 3, (233-246).
- Buratti, F.M. & Testai, E. (2007). Evidences for CYP3A4 autoactivation in the desulfuration of dimethoate by the human liver. *Toxicology*, Vol. 241, No. 1-2, (33-46).
- Buratti, F.M.; D'Aniello, A.; Volpe, M.T.; Meneguz, A. & Testai, E. (2005). Malathion bioactivation in the human liver liver: The contribution of different cytochrome P450 isoform. *Drug Metab. Dispos.*, Vol. 33, No. 3, (295-302).
- Buratti, F.M.; Volpe, M.T.; Meneguz, A.; Vittozzi, L. & Testai, E. (2003). CYP-specific bioactivation of four organophosphorothioate pesticides by human liver microsomes. *Toxicol. Appl. Pharmacol.*, Vol. 186, No. 3, (143-154).
- Buratti, F.M.; Volpe, M.T.; Fabrizi, L.; Meneguz, A.; Vittozzi, L. & Testai, E. (2002). Kinetic parameters of OPT pesticide desulfuration by c-DNA expressed human CYPs. *Environ. Toxicol. Pharmacol.*, Vol. 11, No. 3-4, (181-190).
- Butler, A.M. & Murray, M. (1997). Biotransformation of parathion in human liver: Participation of CYP3A4 and its inactivation during microsomal parathion oxidation. *J. Pharmacol. Exp. Ther.*, Vol. 280, No. 2, (966-973).
- Butler, M.A.; Iwasaki, M.; Guengerich, F.P. & Kadlubar, F.F. (1989). Human cytochrome P-450PA (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci. U. S. A.*, Vol. 86, No. 20, (7696-7700).
- Casabar, R.C.T.; Wallace, A.D.; Hodgson, E. & Rose, R.L. (2006). Metabolism of endosulfan- $\{\alpha\}$ by human liver microsomes and its utility as a simultaneous in vitro probe for CYP2B6 and CYP3A4. *Drug Metab. Dispos.*, Vol. 34, No. 10, (1779-1785).
- Choi, H.; Engene, N.; Smith, J.E.; Preskitt, L.B. & Gerwick, W.H. (2010a). Crossbyanols A-D, toxic brominated polyphenyl ethers from the Hawai'ian bloom-forming Cyanobacterium Leptolyngbya crossbyana. *J. Nat. Prod.*, Vol. 73, No. 4, (517-522).
- Choi, H.; Pereira, A.R.; Cao, Z.; Shuman, C.F.; Engene, N.; Byrum, T.; Matainaho, T.; Murray, T.F.; Mangoni, A. & Gerwick, W.H. (2010b). The hoiamides, structurally intriguing neurotoxic lipopeptides from papua new guinea marine cyanobacteria. *J. Nat. Prod.*, Vol. DOI: 10.1021/np100468n, , (null-null).
- Christopher Gorski, J.; Hall, S.D.; Jones, D.R. & VandenBranden, M. (1994). Regioselective biotransformation of midazolam by members of the human cytochrome P450 3A (CYP3A) subfamily. *Biochem. Pharmacol.*, Vol. 47, No. 9, (1643-1653).
- Code, E.L.; Crespi, C.L.; Penman, B.W.; Gonzalez, F.J.; Chang, T.K.H. & Waxman, D.J. (1997). Human cytochrome P4502B6. interindividual hepatic expression, substrate specificity, and role in procarcinogen activation. *Drug Metab. Dispos.*, Vol. 25, No. 8, (985-993).

- Coleman, S.; Linderman, R.; Hodgson, E. & Rose, R.L. (2000). Comparative metabolism of chloroacetamide herbicides and selected metabolites in human and rat liver microsomes. *Environ. Health Perspect.*, Vol. 108, No. 12, (1151).
- Dalisay, D.S. & Molinski, T.F. (2009). NMR quantitation of natural products at the nanomole scale. *J. Nat. Prod.*, Vol. 72, No. 4, (739-744).
- Dhawan, A.; Parmar, D.; Das, M. & Seth, P.K. (1990). Cytochrome P-450 dependent monooxygenases in neuronal and glial cells: Inducibility and specificity. *Biochem. Biophys. Res. Commun.*, Vol. 170, No. 2, (441-447).
- Ding, X. & Kaminsky, L.S. (2003). Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts. *Annu. Rev. Pharmacol. Toxicol.*, Vol. 43, No. 1, (149-173).
- Djukovic, D.; Appiah-Amponsah, E.; Shanaiah, N.; Gowda, G.A.N.; Henry, I.; Everly, M.; Tobias, B. & Raftery, D. (2008). Ibuprofen metabolite profiling using a combination of SPE/column-trapping and HPLC-micro-coil NMR. *J. Pharm. Biomed. Anal.*, Vol. 47, No. 2, (328-334).
- Doecke, D.J.; Veronese, M.E.; Pond, S.M.; Miners, J.O.; Birkett, D.J.; Sansom, L.M. & McManus, M.E. (1991). Relationship between phenytoin and tolbutamide hydroxylations in human liver microsomes. *Br. J. Clin. Pharmacol.*, Vol. 31, No. 2, (125-130).
- Domanski, T.L.; Finta, C.; Halpert, J.R. & Zaphiropoulos, P.G. (2001). cDNA cloning and initial characterization of CYP3A43, a novel human cytochrome P450. *Mol. Pharmacol.*, Vol. 59, No. 2, (386-392).
- Dutcher, J.S. & Boyd, M.R. (1979). Species and strain differences in target organ alkylation and toxicity by 4-ipomeanol predictive value of covalent binding in studies of target organ toxicities by reactive metabolites. *Biochemical Pharmacology*, Vol. 28, No. 23, (3367-3372).
- Edwards, R.J.; Adams, D.A.; Watts, P.S.; Davies, D.S. & Boobis, A.R. (1998). Development of a comprehensive panel of antibodies against the major xenobiotic metabolising forms of cytochrome P450 in humans. *Biochem. Pharmacol.*, Vol. 56, No. 3, (377-387).
- Ekins, S. & Wrighton, S.A. (1999). The role of CYP2B6 in human xenobiotic metabolism. *Drug Metab. Rev.*, Vol. 31, No. 3, (719-754).
- Ellenhorns, M.J.; Schonwald, S.; Ordog, G. & Wasserberger, J. (1997). *Ellenhorn's medical toxicology: diagnosis and treatment of human poisoning*, Williams & Wilkins, Maryland.
- Eriksson, C. & Brittebo, E.B. (1991). Metabolic activation of the herbicide dichlobenil in the olfactory mucosa of mice and rats. *Chem. Biol. Interact.*, Vol. 79, No. 2, (165-177).
- Faccioliá, G.; Hidestrand, M.; Von Bahr, C. & Tybring, G. (2001). Cytochrome P450 isoforms involved in melatonin metabolism in human liver microsomes. *Eur. J. Clin. Pharmacol.*, Vol. 56, No. 12, (881-888).
- Fang, J.; Coutts, R.T.; McKenna, K.F. & Baker, G.B. (1998). Elucidation of individual cytochrome P450 enzymes involved in the metabolism of clozapine. *Naunyn Schmiedebergs Arch. Pharmacol.*, Vol. 358, No. 5, (592-599).
- Faucette, S.R.; Hawke, R.L.; Lecluyse, E.L.; Shord, S.S.; Yan, B.; Laethem, R.M. & Lindley, C.M. (2000). Validation of bupropion hydroxylation as a selective marker of human cytochrome P450 2B6 catalytic activity. *Drug Metab. Dispos.*, Vol. 28, No. 10, (1222-1230).

- Fountain, S.T. (2002). A Mass Spectrometry Primer, In: *Mass spectrometry in drug discovery*, Rossi, D.T. & Sinz, M.W., (Ed.), (25-84), Marcel Dekker, ISBN: 0-8247-0607-2, New York
- Foxenberg, R.J.; McGarrigle, B.P.; Knaak, J.B.; Kostyniak, P.J. & Olson, J.R. (2007). Human hepatic cytochrome P450-specific metabolism of parathion and chlorpyrifos. *Drug Metab. Dispos.*, Vol. 35, No. 2, (189-193).
- Furuya, H.; Fernandez-Salguero, P.; Gregory, W.; Taber, H.; Steward, A.; Gonzalez, F.J. & Idle, J.R. (1995). Genetic polymorphism of CYP2C9 and its effect on warfarin maintenance dose requirement in patients undergoing anticoagulation therapy. *Pharmacogenetics*, Vol. 5, No. 6, (389-392).
- Galetin, A.; Brown, C.; Hallifax, D.; Ito, K. & Houston, J.B. (2004). Utility of recombinant enzyme kinetics in prediction of human clearance: impact of variability, CYP3A5, and CYP2C19 on CYP3A4 probe substrates. *Drug Metab. Dispos.*, Vol. 32, No. 12, (1411-1420).
- Godin, S.J.; Crow, J.A.; Scollon, E.J.; Hughes, M.F.; DeVito, M.J. & Ross, M.K. (2007). Identification of rat and human cytochrome P450 isoforms and a rat serum esterase that metabolize the pyrethroid insecticides deltamethrin and esfenvalerate. *Drug Metab. Dispos.*, Vol. 35, No. 9, (1664-1671).
- Goldstein, J.A. (2001). Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br. J. Clin. Pharmacol.*, Vol. 52, No. 4, (349-355).
- Goldstein, J.A. & de Morais, S.M.F. (1994). Biochemistry and molecular biology of the human CYP2C subfamily. *Pharmacogenetics*, Vol. 4, No. 6, (285-300).
- Gomez-Lechon, M.J.; Donato, M.T.; Castell, J.V. & Jover, R. (2004). Human hepatocytes in primary culture: the choice to investigate drug metabolism in man. *Curr. Drug Metab.*, Vol. 5, No. 5, (443-462).
- Granfors, M.T.; Backman, J.T.; Neuvonen, M.; Ahonen, J. & Neuvonen, P.J. (2004a). Fluvoxamine drastically increases concentrations and effects of tizanidine: a potentially hazardous interaction. *Clin. Pharmacol. Ther.*, Vol. 75, No. 5, (331-341).
- Granfors, M.T.; Backman, J.T.; Laitila, J. & Neuvonen, P.J. (2004b). Tizanidine is mainly metabolized by cytochrome P450 1A2 in vitro. *Br. J. Clin. Pharmacol.*, Vol. 57, No. 3, (349-353).
- Granvil, C.P.; Madan, A.; Sharkawi, M.; Parkinson, A. & Wainer, I.W. (1999). Role of CYP2B6 and CYP3A4 in the in vitro N-dechloroethylation of (R)- and (S)-ifosfamide in human liver microsomes. *Drug Metab. Dispos.*, Vol. 27, No. 4, (533-541).
- Gray, I.C.; Nobile, C.; Muresu, R.; Ford, S. & Spurr, N.K. (1995). A 2.4-megabase physical map spanning the CYP2C gene cluster on chromosome 10q24. *Genomics*, Vol. 28, No. 2, (328-332).
- Gu, L.; Gonzalez, F.J.; Kalow, W. & Tang, B.K. (1992). Biotransformation of caffeine, paraxanthine, theobromine and theophylline by cDNA-expressed human CYP1A2 and CYP2E1. *Pharmacogenetics*, Vol. 2, No. 2, (73-77).
- Guengerich, F.P. (1995). Cytochromes P450 of human liver. Classification and activity profiles of the major enzymes, In: *Advances in drug metabolism in man*, Pacifici, G.M. & Fracchia, G.N., (Ed.), (179-231), Office for the Official Publications of the European Communities, ISBN: 978-9282739822, Luxembourg
- Guengerich, F.P. (1999). Cytochrome P-450 3A4: regulation and role in drug metabolism. *Annu. Rev. Pharmacol. Toxicol.*, Vol. 39, No. 1, (1-17).

- Guengerich, F.P. & Shimada, T. (1991). Oxidation of toxic and carcinogenic chemicals by human cytochrome P-450 enzymes. *Chem. Res. Toxicol.*, Vol. 4, No. 4, (391-407).
- Guillouzo, A. (1995). Acquisition and use of humanin vitro liver preparations. *Cell Biol. Toxicol.*, Vol. 11, No. 3, (141-145).
- Haas, D.W.; Ribaudo, H.J.; Kim, R.B.; Tierney, C.; Wilkinson, G.R.; Gulick, R.M.; Clifford, D.B.; Hulgan, T.; Marzolini, C. & Acosta, E.P. (2004). Pharmacogenetics of efavirenz and central nervous system side effects: an adult AIDS clinical trials group study. *AIDS*, Vol. 18, No. 18, (2391-2400).
- Haehner, B.; Gorski, J.; Vandenbranden, M.; Wrighton, S.; Janardan, S.; Watkins, P. & Hall, S. (1996). Bimodal distribution of renal cytochrome P450 3A activity in humans. *Mol. Pharmacol.*, Vol. 50, No. 1, (52-59).
- Hakkola, J.; Raunio, H.; Purkunen, R.; Saarikoski, S.; Vähäkangas, K.; Pelkonen, O.; Edwards, R.J.; Boobis, A.R. & Pasanen, M. (2001). Cytochrome P450 3A expression in the human fetal liver: evidence that CYP3A5 is expressed in only a limited number of fetal livers. *Biol. Neonate*, Vol. 80, No. 3, (193-201).
- Hirota, N.; Ito, K.; Iwatsubo, T.; Green, C.E.; Tyson, C.A.; Shimada, N.; Suzuki, H. & Sugiyama, Y. (2001). *In Vitro/in Vivo* scaling of alprazolam metabolism by CYP3A4 and CYP3A5 in humans. *Biopharm. Drug Dispos.*, Vol. 22, No. 2, (53-71).
- Hjelle, J.; Hazelton, G.; Klaassen, C. & Hjelle, J. (1986). Glucuronidation and sulfation in rabbit kidney. *J. Pharmacol. Exp. Ther.*, Vol. 236, No. 1, (150-156).
- Hodgson, E. & Rose, R.L. (2007a). Human metabolic interactions of environmental chemicals. *J. Biochem. Mol. Toxicol.*, Vol. 21, No. 4, (182-186).
- Hodgson, E. & Rose, R.L. (2007b). The importance of cytochrome P450 2B6 in the human metabolism of environmental chemicals. *Pharm. Ther.*, Vol. 113, No. 2, (420-428).
- Hodgson, E. & Rose, R.L. (2005). Human metabolism and metabolic interactions of deployment-related chemicals. *Drug Metab. Rev.*, Vol. 37, No. 1, (1).
- Hoffman, S.M.G.; Fernandez-Salguero, P.; Gonzalez, F.J. & Mohrenweiser, H.W. (1995). Organization and evolution of the cytochrome P450 CYP2A-2B-2F subfamily gene cluster on human chromosome 19. *J. Mol. Evol.*, Vol. 41, No. 6, (894-900).
- Holzgrabe, U. (2010). Quantitative NMR spectroscopy in pharmaceutical applications. , Vol. 57, No. 2, (229-240), 0079-6565
- Honkakoski, P. & Negishi, M. (1997). Characterization of a phenobarbital-responsive enhancer module in mouse P450 cyp2b10 gene. *J. Biol. Chem.*, Vol. 272, No. 23, (14943-14949).
- Huang, W.; Lin, Y.S.; McConn, D.J.II; Calamia, J.C.; Totah, R.A.; Isoherranen, N.; Glodowski, M. & Thummel, K.E. (2004). Evidence of significant contribution from CYP3A5 to hepatic drug metabolism. *Drug Metab. Dispos.*, Vol. 32, No. 12, (1434-1445).
- Huang, Z.; Roy, P. & Waxman, D.J. (2000). Role of human liver microsomal CYP3A4 and CYP2B6 in catalyzing N-dechloroethylation of cyclophosphamide and ifosfamide. *Biochem. Pharmacol.*, Vol. 59, No. 8, (961-972).
- Imaoka, S.; Yamada, T.; Hiroi, T.; Hayashi, K.; Sakaki, T.; Yabusaki, Y. & Funae, Y. (1996). Multiple forms of human P450 expressed in *Saccharomyces cerevisiae*: systematic characterization and comparison with those of the rat. *Biochem. Pharmacol.*, Vol. 51, No. 8, (1041-1050).

- Ingelman-Sundberg, M. (2004). Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J.*, Vol. 5, No. 1, (6-13).
- Ingelman-Sundberg, M.; Oscarson, M. & McLellan, R.A. (1999). Polymorphic human cytochrome P450 enzymes: an opportunity for individualized drug treatment. *Trends Pharmacol. Sci.*, Vol. 20, No. 8, (342-349).
- Jessome, L., Lee & Volmer, A. (2006). Ion suppression: A major concern in mass spectrometry. *LCGC North America*, Vol. 24, No. 5, (7).
- Jiang, Z.; Shu, Y.; Chen, X.; Huang, S.; Zhu, R.; Wang, W.; He, N. & Zhou, H. (2002). The role of CYP2C19 in amitriptyline N-demethylation in Chinese subjects. *Eur. J. Clin. Pharmacol.*, Vol. 58, No. 2, (109-113).
- Jinno, H.; Tanaka-Kagawa, T.; Ohno, A.; Makino, Y.; Matsushima, E.; Hanioka, N. & Ando, M. (2003). Functional characterization of cytochrome P450 2B6 allelic variants. *Drug Metab. Dispos.*, Vol. 31, No. 4, (398-403).
- Johnson, J.A. & Burlew, B.S. (1996). Metoprolol metabolism via cytochrome P4502D6 in ethnic populations. *Drug Metab. Dispos.*, Vol. 24, No. 3, (350-355).
- Jung, F.; Richardson, T.H.; Raucy, J.L. & Johnson, E.F. (1997). Diazepam metabolism by cDNA-expressed human 2C P450s. Identification of P4502C18 and P4502C19 as low K_m diazepam N-demethylases. *Drug Metab. Dispos.*, Vol. 25, No. 2, (133-139).
- Kanu, A.B.; Dwivedi, P.; Tam, M.; Matz, L. & Hill, H.H. (2008). Ion mobility-mass spectrometry. *J. Mass Spectrom.*, Vol. 43, No. 1, (1-22).
- Kappers, W.A.; Edwards, R.J.; Murray, S. & Boobis, A.R. (2001). Diazinon Is Activated by CYP2C19 in Human Liver. *Toxicol. Appl. Pharmacol.*, Vol. 177, No. 1, (68-76).
- Khan, K.K.; He, Y.Q.; Correia, M.A. & Halpert, J.R. (2002). Differential oxidation of mifepristone by cytochromes P450 3A4 and 3A5: selective inactivation of P450 3A4. *Drug Metab. Dispos.*, Vol. 30, No. 9, (985-990).
- Khan, W.A.; Park, S.S.; Gelboin, H.V.; Bickers, D.R. & Mukhtar, H. (1989). Monoclonal antibodies directed characterization of epidermal and hepatic cytochrome P-450 isozymes induced by skin application of therapeutic crude coal tar. *J. Invest. Dermatol.*, Vol. 93, No. 1, (40-45).
- Kirchheimer, J. & Brockmoller, J. (2005). Clinical consequences of cytochrome P450 2C9 polymorphisms. *Clin. Pharmacol. Ther.*, Vol. 77, No. 1, (1-16).
- Kitada, M. & Kamataki, T. (1994). Cytochrome P450 in human fetal liver: significance and fetal-specific expression. *Drug Metab. Rev.*, Vol. 26, No. 1-2, (305-323).
- Kitada, M.; Taneda, M.; Itahashi, K. & Kamataki, T. (1991). Four forms of cytochrome P-450 in human fetal liver: purification and their capacity to activate promutagens. *Cancer Sci.*, Vol. 82, No. 4, (426-432).
- Kitada, M.; Kamataki, T.; Itahashi, K.; Rikihisa, T. & Kanakubo, Y. (1987). Significance of cytochrome P-450 (P-450 HFLa) of human fetal livers in the steroid and drug oxidations. *Biochem. Pharmacol.*, Vol. 36, No. 4, (453-456).
- Kitada, M.; Kamataki, T.; Itahashi, K.; Rikihisa, T.; Kato, R. & Kanakubo, Y. (1985). Purification and properties of cytochrome P-450 from homogenates of human fetal livers. *Arch. Biochem. Biophys.*, Vol. 241, No. 1, (275-280).
- Kitagawa, K.; Kunugita, N.; Katoh, T.; Yang, M. & Kawamoto, T. (1999). The significance of the homozygous CYP2A6 deletion on nicotine metabolism: a new genotyping

- method of CYP2A6 using a single PCR-RFLP. *Biochem. Biophys. Res. Commun.*, Vol. 262, No. 1, (146-151).
- Kobayashi, K.; Chiba, K.; Yagi, T.; Shimada, N.; Taniguchi, T.; Horie, T.; Tani, M.; Yamamoto, T.; Ishizaki, T. & Kuroiwa, Y. (1997). Identification of cytochrome P450 isoforms involved in citalopram N-demethylation by human liver microsomes. *J. Pharmacol. Exp. Ther.*, Vol. 280, No. 2, (927-933).
- Komatsu, T.; Yamazaki, H.; Asahi, S.; Gillam, E.M.J.; Guengerich, F.P.; Nakajima, M. & Yokoi, T. (2000). Formation of a dihydroxy metabolite of phenytoin in human liver microsomes/cytosol: roles of cytochromes P450 2c9, 2c19, and 3a4. *Drug Metab. Dispos.*, Vol. 28, No. 11, (1361-1368).
- Konradsen, F.; van der Hoek, W.; Gunnell, D. & Eddleston, M. (2005). Missing deaths from pesticide self-poisoning at the IFCS Forum IV. *Bull. World Health Organ.*, Vol. 83, No. 2, (157-158).
- Kovacs, H.; Moskau, D. & Spraul, M. (2005). Cryogenically cooled probes-a leap in NMR technology. *Progress in Nuclear Magnetic Resonance Spectroscopy*, Vol. 46, No. 2, (131-155), 0079-6565
- Kremers, P. (1999). Liver microsomes: a convenient tool for metabolism studies but ... In: *European symposium on the prediction of drug metabolism in man: progress and problems*, Boobis, A.R.; Kremers, P.; Pelkonen, O. & Pithan, K., (Ed.), (38-52), Office for Official Publications of the European Communities,
- Kuehl, P.; Zhang, J.; Lin, Y.; Lamba, J.; Assem, M.; Schuetz, J.; Watkins, P.B.; Daly, A.; Wrighton, S.A.; Hall, S.D.; Maurel, P.; Relling, M.; Brimer, C.; Yasuda, K.; Venkataraman, R.; Strom, S.; Thummel, K.; Boguski, M.S. & Schuetz, E. (2001). Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. *Nat. Genet.*, Vol. 27, No. 4, (383).
- Lang, D.H.; Rettie, A.E. & Bocker, R.H. (1997). Identification of enzymes involved in the metabolism of atrazine, terbutylazine, ametryne, and terbutryne in human liver microsomes. *Chem. Res. Toxicol.*, Vol. 10, No. 9, (1037-1044).
- Lang, T.; Klein, K.; Fischer, J.; Nussler, A.K.; Neuhaus, P.; Hofmann, U.; Eichelbaum, M.; Schwab, M. & Zanger, U.M. (2001). Extensive genetic polymorphism in the human CYP2B6 gene with impact on expression and function in human liver. *Pharmacogenetics*, Vol. 11, No. 5, (399-415).
- Lawton, M.; Gasser, R.; Tynes, R.; Hodgson, E. & Philpot, R. (1990). The flavin-containing monooxygenase enzymes expressed in rabbit liver and lung are products of related but distinctly different genes. *J. Biol. Chem.*, Vol. 265, No. 10, (5855-5861).
- LeBlanc, G.A. & Dauterman, W.C. (2001). Conjugation and elimination of toxicants, In: *Introduction to Biochemical Toxicology*, Hodgson, E. & Smart, R.C., (Ed.), Wiley, ISBN: 9780838543320, New York
- Lee, H.; Moon, J.; Chang, C.; Choi, H.; Park, H.; Park, B.; Lee, H.; Hwang, E.; Lee, Y.; Liu, K. & Kim, J. (2006). Stereoselective metabolism of endosulfan by human liver microsomes and human cytochrome P450 isoforms. *Drug Metab. Dispos.*, Vol. 34, No. 7, (1090-1095).
- Lee, S.S.T.; Buters, J.T.M.; Pineau, T.; Fernandez-Salguero, P. & Gonzalez, F.J. (1996). Role of CYP2E1 in the hepatotoxicity of acetaminophen. *J. Biol. Chem.*, Vol. 271, No. 20, (12063-12067).

- Leoni, C.; Buratti, F.M. & Testai, E. (2008). The participation of human hepatic P450 isoforms, flavin-containing monooxygenases and aldehyde oxidase in the biotransformation of the insecticide fenthion. *Toxicol. Appl. Pharmacol.*, Vol. 233, No. 2, (343-352).
- Lewis, D.F.V. (2004). 57 Varieties: the human cytochromes P450. *Pharmacogenomics*, Vol. 5, No. 3, (305-318).
- Li, X.; Bjorkman, A.; Andersson, T.B.; Ridderstrom, M. & Masimirembwa, C.M. (2002). Amodiaquine clearance and its metabolism to N-desethylamodiaquine is mediated by CYP2C8: A new high affinity and turnover enzyme-specific probe substrate. *J. Pharmacol. Exp. Ther.*, Vol. 300, No. 2, (399-407).
- Lin, J.H. & Lu, A.Y.H. (1997). Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol. Rev.*, Vol. 49, No. 4, (403-449).
- Lin, Y.S.; Dowling, A.L.S.; Quigley, S.D.; Farin, F.M.; Zhang, J.; Lamba, J.; Schuetz, E.G. & Thummel, K.E. (2002). Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. *Mol. Pharmacol.*, Vol. 62, No. 1, (162-172).
- Liu, C.; Russell, R.M. & Wang, X.D. (2003). Exposing ferrets to cigarette smoke and a pharmacological dose of beta-carotene supplementation enhance in vitro retinoic acid catabolism in lungs via induction of cytochrome P450 enzymes. *J. Nutr.*, Vol. 133, No. 1, (173-179).
- Liu, Y.; Hao, H.; Liu, C.; Wang, G. & Xie, H. (2007). Drugs as CYP3A probes, inducers, and inhibitors. *Drug Metab. Rev.*, Vol. 39, No. 4, (699).
- Maroni, M.; Fanetti, A.C. & Metruccio, F. (2006). Risk assessment and management of occupational exposure to pesticides in agriculture. *Med Lav*, (430-437).
- Mazur, C.S.; Kenneke, J.F.; Tebes-Stevens, C.; Okino, M.S. & Lipscomb, J.C. (2007). In vitro metabolism of the fungicide and environmental contaminant *trans*-bromuconazole and implications for risk assessment. *J. Toxicol. Environ. Health, A: Curr. Iss.*, Vol. 70, No. 14, (1241-1250).
- McKinnon, R.A.; Hall, P.M.; Quattrochi, L.C.; Tukey, R.H. & McManus, M.E. (1991). Localisation of CYP1A1 and CYP1A2 messenger RNA in normal human liver and in hepatocellular carcinoma by *in situ* hybridization. *Hepatology*, Vol. 14, No. 5, (848-856).
- Messina, E.S.; Tyndale, R.F. & Sellers, E.M. (1997). A major role for CYP2A6 in nicotine C-oxidation by human liver microsomes. *J. Pharmacol. Exp. Ther.*, Vol. 282, No. 3, (1608-1614).
- Miners, J.O. & Birkett, D.J. (1998). Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br. J. Clin. Pharmacol.*, Vol. 45, No. 6, (525-538).
- Musana, A.K. & Wilke, R.A. (2005). Gene-based drug prescribing: clinical implications of the cytochrome P450 genes. *WMJ*, Vol. 104, No. 6, (61-66).
- Mutch, E.; Daly, A.K.; Leathart, J.B.; Blain, P.G. & Williams, F.M. (2003). Do multiple cytochrome P450 isoforms contribute to parathion metabolism in man? *Arch. Toxicol.*, Vol. 77, No. 6, (313-320).
- Mutch, E. & Williams, F.M. (2006). Diazinon, chlorpyrifos and parathion are metabolised by multiple cytochromes P450 in human liver. *Toxicology*, Vol. 224, No. 1-2, (22-32).
- Nagahori, H.; Yoshino, H.; Tomigahara, Y.; Isobe, N.; Kaneko, H. & Nakatsuka, I. (2000). Metabolism of furametpyr. 1. identification of metabolites and in vitro

- biotransformation in rats and humans. *J. Agric. Food Chem.*, Vol. 48, No. 11, (5754-5759).
- Nakajima, M.; Yamamoto, T.; Nunoya, K.; Yokoi, T.; Nagashima, K.; Inoue, K.; Funae, Y.; Shimada, N.; Kamataki, T. & Kuroiwa, Y. (1996a). Characterization of CYP2A6 involved in 3'-hydroxylation of cotinine in human liver microsomes. *J. Pharmacol. Exp. Ther.*, Vol. 277, No. 2, (1010-1015).
- Nakajima, M.; Yamamoto, T.; Nunoya, K.; Yokoi, T.; Nagashima, K.; Inoue, K.; Funae, Y.; Shimada, N.; Kamataki, T. & Kuroiwa, Y. (1996b). Role of human cytochrome P4502A6 in C-oxidation of nicotine. *Drug Metab. Dispos.*, Vol. 24, No. 11, (1212-1217).
- Nelson, D.R.; Zeldin, D.C.; Hoffman, S.M.G.; Maltais, L.J.; Wain, H.M. & Nebert, D.W. (2004). Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes and alternative-splice variants. *Pharmacogenetics*, Vol. 14, No. 1, (1-18).
- Nelson, D.R.; Koymans, L.; Kamataki, T.; Stegeman, J.J.; Feyereisen, R.; Waxman, D.J.; Waterman, M.R.; Gotoh, O.; Coon, M.J.; Estabrook, R.W.; Gunsalus, I.C. & Nebert, D.W. (1996). P450 superfamily: update on new sequences, gene mapping, accession numbers and nomenclature. *Pharmacogenetics*, Vol. 6, No. 1, (1-42).
- Oesch, F.; Herrero, M.E.; Hengstler, J.G.; Lohmann, M. & Arand, M. (2000). Metabolic Detoxification: Implications for Thresholds. *Toxicol Pathol*, Vol. 28, No. 3, (382-387).
- Ohhira, S.; Enomoto, M. & Matsui, H. (2006). In vitro metabolism of tributyltin and triphenyltin by human cytochrome P-450 isoforms. *Toxicology*, Vol. 228, No. 2-3, (171-177).
- Olinga, P.; Meijer, D.K.F.; Slooff, M.J.H. & Groothuis, G.M.M. (1998). Liver slices in in vitro pharmacotoxicology with special reference to the use of human liver tissue. *Toxicol. In Vitro*, Vol. 12, No. 1, (77-100).
- Paine, M.F.; Khalighi, M.; Fisher, J.M.; Shen, D.D.; Kunze, K.L.; Marsh, C.L.; Perkins, J.D. & Thummel, K.E. (1997). Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. *J. Pharmacol. Exp. Ther.*, Vol. 283, No. 3, (1552-1562).
- Pasanen, M. & Pelkonen, O. (1994). The expression and environmental regulation of P450 enzymes in human placenta. *Crit. Rev. Toxicol.*, Vol. 24, No. 3, (211-229).
- Pedro, F.; Susan, H.; Suzanne, C.; Harvey, M.; Hannu, R.; Arja, R.; Olavi, P.; Jin-ding, H.; William, E.; Jeffrey, I. & Frank, G. (1995). A genetic polymorphism in coumarin 7-hydroxylation: sequence of the human CYP2A genes and identification of variant CYP2A6 alleles. *Am. J. Hum. Genet.*, Vol. 57, No. 3, (651-660).
- Pelkonen, O.; Turpeinen, M.; Hakkola, J.; Honkakoski, P.; Hukkanen, J. & Raunio, H. (2008). Inhibition and induction of human cytochrome P450 enzymes: current status. *Arch. Toxicol.*, Vol. 82, No. 10, (667-715).
- Pelkonen, O. & Breimer, D.D. (1994). Role of environmental factors in the pharmacokinetics of drugs: Considerations with respect to animal models, P-450 enzymes, and probe drugs, In: *Handbook of Experimental Pharmacology*, Welling, P.G. & Balant, L.P., (Ed.), (289-332), Springer-Verlag, ISBN: 0387575065, Berlin, Germany
- Pelkonen, O. & Turpeinen, M. (2007). In vitro-in vivo extrapolation of hepatic clearance: Biological tools, scaling factors, model assumptions and correct concentrations. *Xenobiotica*, Vol. 37, No. 10, (1066-1089).

- Pelkonen, O. & Raunio, H. (2005). In vitro screening of drug metabolism during drug development: can we trust the predictions? *Expert Opin. Drug Metab. Toxicol.*, Vol. 1, No. 1, (49-59).
- Pelkonen, O.; Turpeinen, M.; Uusitalo, J.; Rautio, A. & Raunio, H. (2005). Prediction of drug metabolism and interactions on the basis of in vitro investigations. *Basic Clin. Pharmacol. Toxicol.*, Vol. 96, No. 3, (167-175).
- Pelkonen, O.; Rautio, A.; Raunio, H. & Pasanen, M. (2000). CYP2A6: a human coumarin 7-hydroxylase. *Toxicology*, Vol. 144, No. 1-3, (139-147).
- Peter, R.; Bocker, R.; Beaune, P.H.; Iwasaki, M.; Guengerich, F.P. & Yang, C.S. (1990). Hydroxylation of chlorzoxazone as a specific probe for human liver cytochrome P-450IIE1. *Chem. Res. Toxicol.*, Vol. 3, No. 6, (566-573).
- Peters, W.H.M. & Kremers, P.G. (1989). Cytochromes P-450 in the intestinal mucosa of man. *Biochemical Pharmacology*, Vol. 38, No. 9, (1535-1538).
- Rahman, A.; Korzekwa, K.R.; Grogan, J.; Gonzalez, F.J. & Harris, J.W. (1994). Selective biotransformation of taxol to 6{alpha}-hydroxytaxol by human cytochrome P450 2C8. *Cancer Res.*, Vol. 54, No. 21, (5543-5546).
- Raucy, J.L.; Kraner, J.C. & Lasker, J.M. (1993). Bioactivation of halogenated hydrocarbons by cytochrome P4502E1. *Crit. Rev. Toxicol.*, Vol. 23, No. 1, (1).
- Raunio, H.; Pasanen, M.; Mäenpää, J.; Hakkola, J. & Pelkonen, O. (1995). Expression of extrahepatic cytochrome P450 in humans, In: *Advances in drug metabolism in man*, Pacifici, G.M. & Fracchia, G.N., (Ed.), (234-287), ISBN: 92-827-3982-1, Luxembourg
- Rendic, S. & Di Carlo, F.J. (1997). Human cytochrome P450 enzymes: A status report summarizing their reactions, substrates, inducers, and inhibitors. *Drug Metab. Rev.*, Vol. 29, No. 1-2, (413-580).
- Rettie, A.E. & Jones, J.P. (2005). Clinical and toxicological relevance of CYP2C9: drug-drug interactions and pharmacogenetics. *Annu. Rev. Pharmacol. Toxicol.*, Vol. 45, No. 1, (477-494).
- Rettie, A.E.; Korzekwa, K.R.; Kunze, K.L.; Lawrence, R.F.; Eddy, A.C.; Aoyama, T.; Gelboin, H.V.; Gonzalez, F.J. & Trager, W.F. (1992). Hydroxylation of warfarin by human cDNA-expressed cytochrome P-450: a role for P-4502C9 in the etiology of (S)-warfarin-drug interactions. *Chem. Res. Toxicol.*, Vol. 5, No. 1, (54-59).
- Richardson, T.H.; Griffin, K.J.; Jung, F.; Raucy, J.L. & Johnson, E.F. (1997). Targeted anti peptide antibodies to cytochrome P450 2C18 based on epitope mapping of an inhibitory monoclonal antibody to P450 2C51. *Arch. Biochem. Biophys.*, Vol. 338, No. 2, (157-164).
- Rodrigues, A.D. (1999). Integrated cytochrome P450 reaction phenotyping. Attempting to bridge the gap between cDNA-expressed cytochromes P450 and native human liver microsomes. *Biochem. Pharmacol.*, Vol. 57, No. 5, (465-480).
- Rose, R.L. & Hodgson, E. (2004). Metabolism of toxicants, In: *Text Book of Modern Toxicology*, Hodgson, E., (Ed.), (111-148), Wiley, ISBN: 978-0-470-46206-5, New York
- Rossi, D.T. (2002). Sample Preparation and Handling for LC/MS in Drug Discovery, In: *Mass spectrometry in drug discovery*, Rossi, D.T. & Sinz, M.W., (Ed.), (171-214), Marcel Dekker, ISBN: 0-8247-0607-2, New York
- Rostami-Hodjegan, A. & Tucker, G.T. (2007). Simulation and prediction of in vivo drug metabolism in human populations from in vitro data. *Nat. Rev. Drug Discov.*, Vol. 6, No. February, (140-148).

- Roy, P.; Tretyakov, O.; Wright, J. & Waxman, D.J. (1999a). Stereoselective metabolism of ifosfamide by human P-450s 3A4 and 2B6. Favorable metabolic properties of R-enantiomer. *Drug Metab. Dispos.*, Vol. 27, No. 11, (1309-1318).
- Roy, P.; Yu, L.J.; Crespi, C.L. & Waxman, D.J. (1999b). Development of a substrate-activity based approach to identify the major human liver P-450 catalysts of cyclophosphamide and ifosfamide activation based on cDNA-expressed activities and liver microsomal P-450 profiles. *Drug Metab. Dispos.*, Vol. 27, No. 6, (655-666).
- Salonpää, P.; Hakkola, J.; Pasanen, M.; Pelkonen, O.; Vähäkangas, K.; Battula, N.; Nouso, K. & Raunio, H. (1993). Retrovirus-mediated stable expression of human CYP2A6 in mammalian cells. *Eur. J. Pharmacol.*, Vol. 248, No. 2, (95-102).
- Sams, C.; Mason, H.J. & Rawbone, R. (2000). Evidence for the activation of organophosphate pesticides by cytochromes P450 3A4 and 2D6 in human liver microsomes. *Toxicol. Lett.*, Vol. 116, No. 3, (217-221).
- Sarkar, M.A. & Jackson, B.J. (1994). Theophylline N-demethylations as probes for P4501A1 and P4501A2. *Drug Metab. Dispos.*, Vol. 22, No. 6, (827-834).
- Schuetz, J.D.; Beach, D.L. & Guzelian, P.S. (1994). Selective expression of cytochrome P450 CYP3A mRNAs in embryonic and adult human liver. *Pharmacogenetics*, Vol. 4, No. 1, (11-20).
- Schulz-Jander, D.A. & Casida, J.E. (2002). Imidacloprid insecticide metabolism: human cytochrome P450 isozymes differ in selectivity for imidazolidine oxidation versus nitroimine reduction. *Toxicol. Lett.*, Vol. 132, No. 1, (65-70).
- Scallon, E.J.; Starr, J.M.; Godin, S.J.; DeVito, M.J. & Hughes, M.F. (2009). In vitro metabolism of pyrethroid pesticides by rat and human hepatic microsomes and cytochrome P450 isoforms. *Drug Metab. Dispos.*, Vol. 37, No. 1, (221-228).
- Scott, E.E. & Halpert, J.R. (2005). Structures of cytochrome P450 3A4. *Trends Biochem. Sci.*, Vol. 30, No. 1, (5-7).
- Sesardic, D.; Boobis, A.R.; Murray, B.P.; Murray, S.; Segura, J.; de la Torre, R. & Davis, D.S. (1990). Furanylline is a potent and selective inhibitor of cytochrome P4501A2 in man. *Br. J. Clin. Pharmacol.*, Vol. 29, No. 5, (651-663).
- Sheehan, T.L. (2002). The best MS option: GC-MS and LC-MS. *Am. Lab.*, Vol. 34, No. 18, (40-43).
- Shimada, T.; Yamazaki, H. & Guengerich, F.P. (1996). Ethnic-related differences in coumarin 7-hydroxylation activities catalyzed by cytochrome P4502A6 in liver microsomes of Japanese and Caucasian populations. *Xenobiotica*, Vol. 26, No. 4, (395-403).
- Shimada, T.; Yamazaki, H.; Mimura, M.; Inui, Y. & Guengerich, F.P. (1994). Interindividual variations in human liver cytochrome P-450 enzymes involved in the oxidation of drugs, carcinogens and toxic chemicals: studies with liver microsomes of 30 Japanese and 30 Caucasians. *J. Pharmacol. Exp. Ther.*, Vol. 270, No. 1, (414-423).
- Skett, P.; Tyson, C.; Guillouzo, A. & Maier, P. (1995). Report on the international workshop on the use of human in vitro liver preparations to study drug metabolism in drug development. *Biochem. Pharmacol.*, Vol. 50, No. 2, (280-285).
- Smith, G.B.J.; Bend, J.R.; Bedard, L.L.; Reid, K.R.; Petsikas, D. & Massey, T.E. (2003). Biotransformation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in peripheral human lung microsomes. *Drug Metab. Dispos.*, Vol. 31, No. 9, (1134-1141).

- Soars, M.G.; Grime, K. & Riley, R.J. (2006). Comparative analysis of substrate and inhibitor interactions with CYP3A4 and CYP3A5. *Xenobiotica*, Vol. 36, No. 4, (287-299).
- Stresser, D.M. & Kupfer, D. (1999). Monospecific Antipeptide Antibody to Cytochrome P-450 2B6. *Drug Metab. Dispos.*, Vol. 27, No. 4, (517-525).
- Stresser, D.M. & Kupfer, D. (1998). Human cytochrome P450-catalyzed conversion of the proestrogenic pesticide methoxychlor into an estrogen. Role of CYP2C19 and CYP1A2 in O-demethylation. *Drug Metab. Dispos.*, Vol. 26, No. 9, (868-874).
- Stubbins, M.J.; Harries, L.W.; Smith, G.; Tarbit, M.H. & Wolf, C.R. (1996). Genetic analysis of the human cytochrome P450 CYP2C9 locus. *Pharmacogenetics*, Vol. 6, No. 5, (429-439).
- Sullivan-Klose, T.H.; Ghanayem, B.I.; Bell, D.A.; Zhang, Z.Y.; Kaminsky, L.S.; Shenfield, G.M.; Miners, J.O.; Birkett, D.J. & Goldstein, J.A. (1996). The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics*, Vol. 6, No. 4, (341-349).
- Tang, J.; Cao, Y.; Rose, R.L. & Hodgson, E. (2002). In vitro metabolism of carbaryl by human cytochrome P450 and its inhibition by chlorpyrifos. *Chem. Biol. interact.*, Vol. 141, No. 3, (229-241).
- Tang, J.; Cao, Y.; Rose, R.L.; Brimfield, A.A.; Dai, D.; Goldstein, J.A. & Hodgson, E. (2001). Metabolism of chlorpyrifos by human cytochrome P450 isoforms and human, mouse, and rat liver microsomes. *Drug Metab. Dispos.*, Vol. 29, No. 9, (1201-1204).
- Tang, W. & Stearns, R.A. (2001). Heterotropic cooperativity of cytochrome P450 3A4 and potential drug-drug interactions. *Curr. Drug Metab.*, Vol. 2, No. 2, (185).
- Tassaneeyakul, W.; Mohamed, Z.; Birkett, D.J.; McManus, M.E.; Veronese, M.E.; Tukey, R.H.; Quattrochi, L.C.; Gonzalez, F.J. & Miners, J.O. (1992). Caffeine as a probe for human cytochromes P450: validation using cDNA-expression, immunoinhibition and microsomal kinetic and inhibitor techniques. *Pharmacogenetics*, Vol. 2, No. 4, (173-183).
- Thummel, K.; Kharasch, E.; Podoll, T. & Kunze, K. (1993). Human liver microsomal enflurane defluorination catalyzed by cytochrome P-450 2E1. *Drug Metab. Dispos.*, Vol. 21, No. 2, (350-357).
- Tjia, J.F.; Colbert, J. & Back, D.J. (1996). Theophylline metabolism in human liver microsomes: inhibition studies. *J. Pharmacol. Exp. Ther.*, Vol. 276, No. 3, (912-917).
- Tremaine, L.M.; Diamond, G.L. & Quebbemann, A.J. (1985). Quantitative determination of organ contribution to excretory metabolism. *Journal of Pharmacological Methods*, Vol. 13, No. 1, (9-35).
- Tsao, C.; Wester, M.R.; Ghanayem, B.; Coulter, S.J.; Chanas, B.; Johnson, E.F. & Goldstein, J.A. (2001). Identification of human CYP2C19 residues that confer S-mephenytoin 4'-hydroxylation activity to CYP2C9. *Biochemistry*, Vol. 40, No. 7, (1937-1944).
- Turpeinen, M.; Ghiciuc, C.; Opritou, M.; Tursas, L.; Pelkonen, O. & Pasanen, M. (2007). Predictive value of animal models for human cytochrome P450 (CYP)-mediated metabolism: A comparative study in vitro. *Xenobiotica*, Vol. 37, No. 12, (1367-1377).
- Turpeinen, M.; Raunio, H. & Pelkonen, O. (2006). The functional role of CYP2B6 in human drug metabolism: substrates and inhibitors in vitro, in vivo and in silico. *Curr. Drug Metab.*, Vol. 7, No. 7, (705-714).
- Usmani, K.A.; Karoly, E.D.; Hodgson, E. & Rose, R.L. (2004). In vitro sulfoxidation of thioether compounds by human cytochrome P450 and flavin-containing

- monooxygenase isoforms with particular reference to the CYP2C subfamily. *Drug Metab. Dispos.*, Vol. 32, No. 3, (333-339).
- Usmani, K.A.; Rose, R.L.; Goldstein, J.A.; Taylor, W.G.; Brimfield, A.A. & Hodgson, E. (2002). In vitro human metabolism and interactions of repellent N,N-diethyl-m-toluamide. *Drug Metab. Dispos.*, Vol. 30, No. 3, (289-294).
- Venkatakrishnan, K.; Moltke, L.L.V. & Greenblatt, D.J. (1998). Human cytochromes P450 mediating phenacetin O-deethylation in vitro: validation of the high affinity component as an index of CYP1A2 activity. *J. Pharm. Sci.*, Vol. 87, No. 12, (1502-1507).
- Volotinen, M.; Turpeinen, M.; Tolonen, A.; Uusitalo, J.; Maenpaa, J. & Pelkonen, O. (2007). Timolol metabolism in human liver microsomes is mediated principally by CYP2D6. *Drug Metab. Dispos.*, Vol. 35, No. 7, (1135-1141).
- von Bahr, C.; Ursing, C.; Yasui, N.; Tybring, G.; Bertilsson, L. & Röjdmark, S. (2000). Fluvoxamine but not citalopram increases serum melatonin in healthy subjects - an indication that cytochrome P450 CYP1A2 and CYP2C19 hydroxylate melatonin. *Eur. J. Clin. Pharmacol.*, Vol. 56, No. 2, (123-127).
- von Moltke, L.L.; Greenblatt, D.J.; Giancarlo, G.M.; Granda, B.W.; Harmatz, J.S. & Shader, R.I. (2001). Escitalopram (S-citalopram) and its metabolites in vitro: cytochromes mediating biotransformation, inhibitory effects, and comparison to R-citalopram. *Drug Metab. Dispos.*, Vol. 29, No. 8, (1102-1109).
- Wei, C.; Caccavale, R.J.; Weyand, E.H.; Chen, S. & Iba, M.M. (2002). Induction of CYP1A1 and CYP1A2 expressions by prototypic and atypical inducers in the human lung. *Cancer Lett.*, Vol. 178, No. 1, (25-36).
- Wei, C.; Caccavale, R.J.; Kehoe, J.J.; Thomas, P.E. & Iba, M.M. (2001). CYP1A2 is expressed along with CYP1A1 in the human lung. *Cancer Lett.*, Vol. 171, No. 1, (113-120).
- WHO/UNEP. (1990). Public health impact of pesticides used in agriculture, In: World Health Organization and United Nations Environment Programme, (Ed.), , Geneva
- Wijnen, P.A.H.M.; Buijsch, R.A.M.O.D.; Drent, M.; Kuipers, P.M.J.C.; Neef, C.; Bast, A.; Bekers, O. & Koek, G.H. (2007). Review article: the prevalence and clinical relevance of cytochrome P450 polymorphisms. *Aliment. Pharmacol. Ther.*, Vol. 26, No. s2, (211-219).
- Williams, J.A.; Ring, B.J.; Cantrell, V.E.; Jones, D.R.; Eckstein, J.; Ruterbories, K.; Hamman, M.A.; Hall, S.D. & Wrighton, S.A. (2002). Comparative metabolic capabilities of CYP3A4, CYP3A5, and CYP3A7. *Drug Metab. Dispos.*, Vol. 30, No. 8, (883-891).
- Wrighton, S.A.; Stevens, J.C.; Becker, G.W. & Vandenbranden, M. (1993). Isolation and characterization of human liver cytochrome P450 2C19: correlation between 2C19 and S-mephenytoin 4'-hydroxylation. *Arch. Biochem. Biophys.*, Vol. 306, No. 1, (240-245).
- Yamano, S.; Nhamburo, P.T.; Aoyama, T.; Meyer, U.A.; Inaba, T.; Kalow, W.; Gelboin, H.V.; McBride, O.W. & Gonzalez, F.J. (1989). cDNA cloning and sequence and cDNA-directed expression of human P450 IIB1: identification of a normal and two variant cDNAs derived from the CYP2B locus on chromosome 19 and differential expression of the IIB mRNAs in human liver. *Biochemistry*, Vol. 28, No. 18, (7340-7348).

- Yamazaki, H.; Inoue, K.; Hashimoto, M. & Shimada, T. (1999). Roles of CYP2A6 and CYP2B6 in nicotine C-oxidation by human liver microsomes. *Arch. Toxicol.*, Vol. 73, No. 2, (65-70).
- Yasar, Ü.; Eliasson, E.; Dahl, M.; Johansson, I.; Ingelman-Sundberg, M. & Sjöqvist, F. (1999). Validation of methods for CYP2C9 genotyping: frequencies of mutant alleles in a swedish population. *Biochem. Biophys. Res. Commun.*, Vol. 254, No. 3, (628-631).
- Yun, C.H.; Shimada, T. & Guengerich, F.P. (1991). Purification and characterization of human liver microsomal cytochrome P-450 2A6. *Mol. Pharmacol.*, Vol. 40, No. 5, (679-685).
- Zamek-Gliszczynski, M.J.; Hoffmaster, K.A.; Nezasa, K.; Tallman, M.N. & Brouwer, K.L.R. (2006). Integration of hepatic drug transporters and phase II metabolizing enzymes: mechanisms of hepatic excretion of sulfate, glucuronide, and glutathione metabolites. *Eur. J. Pharm. Sci.*, Vol. 27, No. 5, (447-486).
- Zanger, U.; Raimundo, S. & Eichelbaum, M. (2004). Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch. Pharmacol.*, Vol. 369, No. 1, (23-37).



Fungicides

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Plant and plant products are affected by a large number of plant pathogens among which fungal pathogens. These diseases play a major role in the current deficit of food supply worldwide. Various control strategies were developed to reduce the negative effects of diseases on food, fiber, and forest crops products. For the past fifty years fungicides have played a major role in the increased productivity of several crops in most parts of the world. Although fungicide treatments are a key component of disease management, the emergence of resistance, their introduction into the environment and their toxic effect on human, animal, non-target microorganisms and beneficial organisms has become an important factor in limiting the durability of fungicide effectiveness and usefulness. This book contains 25 chapters on various aspects of fungicide science from efficacy to resistance, toxicology and development of new fungicides that provides a comprehensive and authoritative account for the role of fungicides in modern agriculture.

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51000 Rijeka, Croatia
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InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
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中国上海市延安西路65号上海国际贵都大酒店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

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