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*Perspectives - Minireview*

# Human Cytochrome P450 Metabolic Activation in Chemical Toxicity

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Cytochrome P450 (P450) enzymes are the major catalysts involved in the biotransformation of various drugs, pollutants, carcinogens, and many endogenous compounds. Most of chemical carcinogens are not active by themselves but they require metabolic activation. P450 isozymes play a pivotal role in the metabolic activation. The activation of arylamines and heterocyclic arylamines (HAAs) involves critical *N*-hydroxylation, usually by P450. CYP1A2 plays an important role in these reactions. Broad exposure to many of these compounds might cause carcinogenicity in animals and humans. On the other hand, P450s can be also involved in the bioactivation of other chemicals including alcohols, aflatoxin B1, acetaminophen, and trichloroethylene, both in humans and in experimental animals. Understanding the P450 metabolic activation of many chemicals is necessary to develop rational strategies for prevention of their toxicities in human health. An important part is the issues of extrapolation between species in predicting risks and variation of P450 enzyme activities in humans.

***Key words***: Cytochrome P450, Metabolic activation, Hydroxylamine, Arylamine, *N*-Acetyltransferase.

## INTRODUCTION

Many chemicals of concern tend to be relatively inert and require enzymatic activation before they can undergo the effective toxic or carcinogenic reactions in the human body. Some of the key studies leading to the discovery of the cytochrome P450 (P450) enzymes were related to the enzymatic activation of toxic and carcinogenic chemicals (Guengerich, 1997a). As the characterization of P450 enzymes has progressed, information about their roles in the overall process of metabolic activation has significantly accumulated.

As early as in 1939, Widmark demonstrated that the extracts of fried horse meat induced cancer when applied to mouse skin (Widmark, 1939). Sugimura and his colleagues showed that the smoke condensate produced by broiling fish and meat was highly mutagenic in *Salmonella typhimurium* test systems (Sugimura *et al.*, 1977). But, the concept of metabolic- or bio-activation of chemicals was not perceived until then. The bioactivation of chemicals to reactive electrophiles was first demonstrated and extensively studied by Miller and Miller (Miller and Miller, 1981; Guengerich, 2001). Their early work demonstrated the ability of chemicals to modulate the metabolism of carcinogens, a phenomenon now described as enzyme induction.

Guengerich's group was able to purify and characterize some of rat and human P450 enzymes, which were involved in specific activation and detoxification of some carcinogens and other toxic chemicals (Guengerich, 2001). P450s are generally considered to be the primary catalysts in the biotransformation of xenobiotic chemicals. The chemistry of P450 bioactivation is reasonably well understood, but the mechanisms underlying biological responses are not. This review will focus on issues in the role and mechanism of human P450s for the metabolic activation of toxic chemicals.

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## METABOLIC ACTIVATION

***Arylamines and heterocyclic arylamines (HAAs).*** Arylamines and HAAs are formed as a consequence of pyrolysis of amino acids or protein-containing foods and their structures were determined in early studies (Nagao *et al.*, 1978; Sugimura, 1978; Sugimura, 1986; Jagerstad *et al.*, 1986; Hatch and Felton, 1986) (Fig. 1). These compounds require bioactivation to be mutagenic or carcinogenic and the detailed metabolic activation mech-

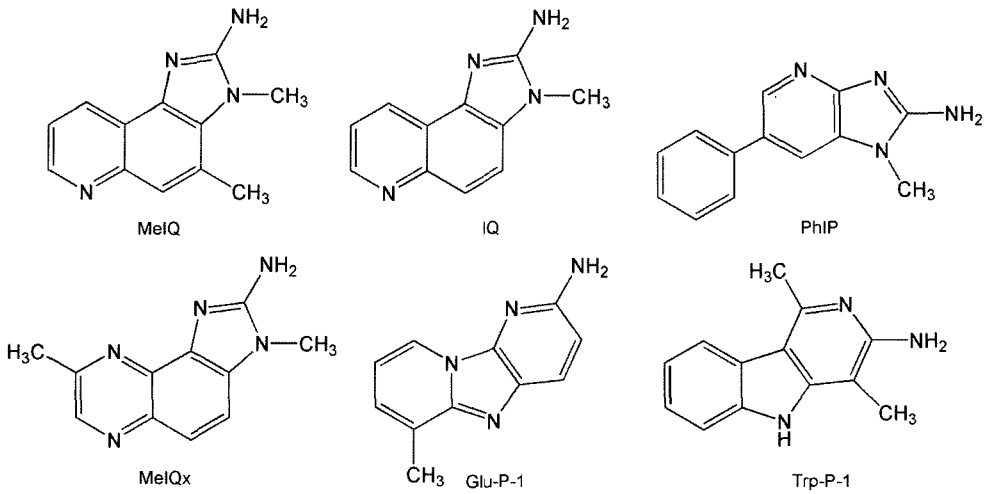


**Fig. 1.** Chemical structures of heterocyclic arylamines found in food.

anism of arylamines and HAAs has been described previously (Kadlubar and Hammons, 1987; Kim and Guengerich, 2005). The major metabolic pathway is *N*-oxidation, which is mediated primarily by P450 enzymes (Frederick *et al.*, 1982; Hammons *et al.*, 1985; Yamazoe *et al.*, 1985). The resulting *N*-hydroxylamine products can be further activated to produce highly reactive ester derivatives that bind covalently to DNA. *N*-acetyltransferase (NAT) enzyme system was considered to be a major secondary activation step in mammals (Kato, 1986). NAT-catalyzed acetylation of *N*-hydroxy-HAAs and arylamines enhanced the genotoxic activity and DNA adduct levels through formation of reactive *N*-acetoxyl esters (Yamazaki *et al.*, 1992; Snyderwine *et al.*, 1993; Oda *et al.*, 1995). The *N*-hydroxy HAAs may react directly with DNA (Frederick *et al.*, 1982). However, the reaction was facilitated when the reactive ester derivatives undergo heterocyclic cleavage to yield reactive aryl nitrenium ion species, which preferentially react to form DNA adducts (Fig. 2). Arylamines and HAAs yield adducts primarily with guanine at the $N^2$ and $C^8$ atoms (Kadlubar *et al.*, 1980).

The human P450 isozymes involved in the metabolism of arylamines, HAAs, and other chemical carcinogens have long been a subject of interest. Some efforts had been made at analysis with early preparations of human P450s (Wang *et al.*, 1983). Analysis of the animal models and subsequent correlations of hepatic expression levels with the *N*-hydroxylation of 4-aminobiphenyl (ABP) (Butler *et al.*, 1989a) led to the view that the enzyme, later known as $P450_{PA}$, has a major role in the *N*-hydroxylation of many arylamines and HAAs. Further evidence followed, with the demonstration that the same enzyme is involved in caffeine $N^3$-demethylation (Butler *et al.*, 1989b) and also many HAA activations can be attributed to this enzyme (Shimada *et al.*, 1989). Phenacetin metabolism had been studied in humans *in vivo*, and the characterization of human CYP1A2 led to insight into the inducibility of CYP1A2 in humans (Pantuck *et al.*, 1974; Distlerath *et al.*, 1985).

The roles of human P450s in the bioactivation of arylamines and HAAs have been considerably documented (Kim and Guengerich, 2005). CYP1A1 and 1A2 have been generally recognized to be the major forms involved in the bioactivation of arylamines and HAAs in human liver and lung microsomes. A representative study with HAAs is presented in Table 1. The findings with CYP1A2 have been confirmed *in vivo* in human

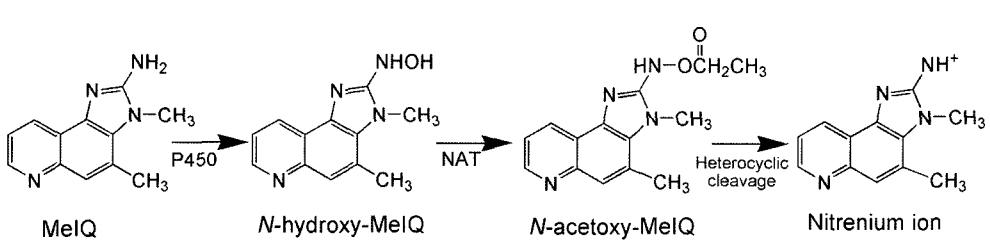


**Fig. 2.** General pathway for metabolic activation of heterocyclic arylamines, as shown for MeIQ.

**Table 1.** Activation of HAAs and arylamines by recombinant P450 in *S. typhimurium*-based genotoxicity system[a]

| HAA | Concentration, μM | Activity (*umu*/min/nmol P450) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 1A1 | 1A2 | 1B1 | 2C9 | 2D6 | 2E1 | 3A4 |
| Glu-P-1 | 1.5 | 27 | 91 | 0 | 4 | 10 | 13 | 7 |
| PhIP | 220 | 7 | 24 | 2 | 4 | 3 | 5 | 4 |
| MeIQx | 0.3 | 16 | 442 | 4 | 0 | 9 | 0 | 0 |
| MeIQ | 0.05 | 24 | 179 | 4 | 0 | 1 | 0 | 4 |
| IQ | 0.5 | 10 | 214 | 7 | 0 | 1 | 9 | 1 |
| Trp-P-1 | 4 | 536 | 321 | 232 | - | - | - | 179 |
| Trp-P-2 | 4 | 578 | 39 | 138 | - | - | - | 555 |
| 2-AA | 0.1 | 90 | 374 | 50 | 0 | 1 | 23 | 0 |
| 2-AF | 12.5 | 46 | 676 | 22 | 0 | 0 | 13 | 25 |

[a](Kim and Guengerich, 2005).

studies, using PhIP and MeIQx. The CYP1A2-selective inhibitor furafylline blocked most of the *in vivo* elimination in human volunteers consumed burned meat (Boobis *et al.*, 1994). Another P450 family 1 member, CYP1B1, has been also shown to be an important enzyme involved in the activation of HAAs as well as development of human cancers (Table 1) (Shimada, 1996). It should be emphasized that some of the P450s (other than family 1) do have measurable activity with some of the substrates, both arylamines and HAAs. In contrast to other arylamines, MOCA *N*-hydroxylation was shown to be preferentially catalyzed by CYP3A4 in human liver (Yun *et al.*, 1992). It has been reported that CYP3A7 can activate some HAAs to mutagens in fetal liver, where P450 1A2 is not expressed (Kitada *et al.*, 1991).

The point should be made that we have treated CYP1A2 (and other P450s) only in terms of the "wild-type" (or more correctly, the predominant) allele thus far. The possibility exists that some individuals have variants that provide unusual catalytic properties. For instance, specific screening systems involving the activation of MeIQ to a genotoxic *N*-hydroxylamine were developed to identify CYP1A2 mutants with high activity in laboratory-generated random libraries (Parikh *et al.*, 1999; Kim and Guengerich 2004). Some of these variants showed 12-fold higher activities of *N*-hydroxylation than wild-type. Other known allelic variants had catalytic efficiencies ($k_{cat}/K_m$) for *N*-hydroxylation of several HAAs within a 3-fold range, although one CYP 1A2 variant failed to incorporate heme and was inactive (Zhou *et al.*, 2004).

A study with CYP1A2-null mice also indicated that CYP1A2 plays an important role in DNA adduct formation with PhIP and IQ *in vivo* (Snyderwine *et al.*, 2002). Differences due to the absence/presence of CYP1A2 were seen in liver, kidney, and colon but not in mammary glands. However, a neonatal bioassay study with CYP1A2 null-mice suggests that an unknown pathway unrelated to CYP1A2 appears to be responsible for the carcinogenesis of PhIP (Kimura *et al.*, 2003).

Interspecies differences in metabolism of HAAs by rat and human CYP1A2 were found in the metabolism of MeIQx and PhIP (Turesky *et al.*, 1998). Although rat and human CYP1A2 have 75% amino acid sequence identity, relatively high levels of CYP1A2 expression in human liver and catalytic activities for HAA *N*-hydroxylation compared to the rat CYP1A2 were observed (Guengerich, 1997b). Important differences between human and rat CYP1A2 were also found in the $C^8$- and *N*-oxidation of MeIQx (Langouët *et al.*, 2001). These suggest that the interspecies differences in P450 enzyme expression and catalytic activities might be significant and must be carefully considered when human health risk was assessed.

The carcinogenicity of IQ, MeIQ, and PhIP was examined in cynomolous monkeys, up to 7 years of administration (Adamson, 2000). IQ and PhIP were potent liver carcinogens and they formed high levels of DNA adducts in a number of organs, particularly the liver, kidney, and heart. However, low mutagenic and carcinogenic activation of MeIQx was observed in this species. Poor activation of MeIQx was explained by the lack of constitutive expression of CYP1A2 and an inability of other P450s to hydroxylate these quinoxalines (Snyderwine *et al.*, 1997).

***Oxidative stress.*** Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. It is a major issue with drug able to make redox cycling such as daunorubicin and also an important component of the toxicity caused by most chemicals that are activated to electrophiles (Guengerich, 2006). Electrophiles deplete reduced glutathione, one of the defenses against damage from reactive oxygen spe-

cies. The contribution of P450 in oxidative stress is complex. $H_2O_2$ and $O_2^{\cdot-}$ are formed in the uncoupled reactions catalyzed by microsomes and purified P450s. Induction and heterologous expression of mammalian P450s lead to oxidative damage in cell culture systems (Park *et al.*, 1996; Cederbaum *et al.*, 2001). Enhanced lipid peroxidation is associated with some P450 substrates but prevented by others. Few studies have been carried out to correlate the oxidative stress indicators with P450s *in vivo*, and some possibilities were existed with biomarkers in NADPH-P450 reductase-deficient transgenic mice (Henderson *et al.*, 2003; Gu *et al.*, 2003).

Alcohol-induced oxidative stress is the result of the combined impairment of antioxidant defense and the production of reactive oxygen species by the mitochondrial electron transport chain, the alcohol-inducible CYP2E1 and activated phagocytes (Albano, 2006; Cederbaum, 2006). Furthermore, hydroxyethyl free radicals (HER) are also generated during ethanol metabolism by CYP2E1. The mechanisms by which oxidative stress contributes to alcohol toxicity are still not completely understood. However, induction of CYP2E1 is a central pathway by which ethanol generates oxidative stress and CYP2E1 metabolizes many other toxic compounds. The toxicity of these agents was enhanced by ethanol, due to induction of CYP2E1 (Cederbaum, 2006).

***Acetaminophen.*** Acetaminophen (Tylenol) has been studied considerably over the years for its metabolic activation. It is widely used as an analgesic and is generally quite safe unless a great overdose occurs, being extensively metabolized by sulfation and glucuronide formation (Guengerich, 1997a). Activation involves oxidation to the iminoquinone, a Michael acceptor that can react with nucleophilic sulfhydryls (Fig. 3). This oxidation has been shown to be catalyzed by human CYP1A2, 2E1, and 3A4 enzymes (Guengerich, 1997a). One mechanism for the toxicity is the reactivity of the iminoquinone with critical protein sulfhydryl groups. Alternatively, the iminoquinone can be reduced back to acetaminophen, thus consuming glutathione or reduced pyridine nucleotides and creating an oxidized environment (Fig. 3). P450s are also able to catalyze the one-electron oxidation of acetaminophen to semiquinone level. This intermediate species would react with $O_2$ to generate $O_2^{\cdot-}$ and initiate events related to oxidative stress previously addressed (Guengerich and Liebler, 1985; Guengerich, 1997a).

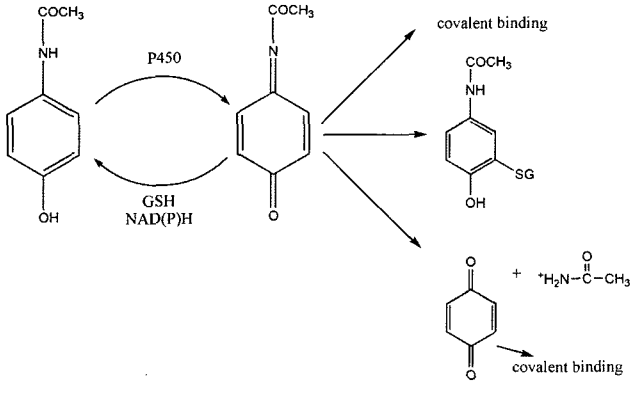


**Fig. 3.** Metabolism of acetaminophen by P450s.

***Aflatoxin B1.*** Aflatoxin $B_1$ ($AFB_1$) provides an interesting case history in issues involved in the metabolism and reactivity of a procarcinogen. It was originally discovered through an incident with livestock, the poisoning of turkeys in UK by mold-contaminated peanut meal (Groopman *et al.*, 1988; Guengerich, 2001). It is a potent hepatocarcinogenic mycotoxin in experimental animals and a hazard to human health. $AFB_1$ is activated to $AFB_1$ *exo*-8,9-epoxide primarily by P450 enzymes, particularly CYP3A4 (Fig. 4). P450s also oxidize $AFB_1$ to deactivated products that are generally poor substrate for epoxidation or, after epoxidation, did not interact with DNA (Guengerich *et al.*, 1998). Other P450s, such as 1A2, are readily able to oxidize $AFB_1$ to $AFM_1$, $AFQ_1$, and $AFB_1$ *endo*-8,9-epoxide (Guengerich *et al.*, 1998).

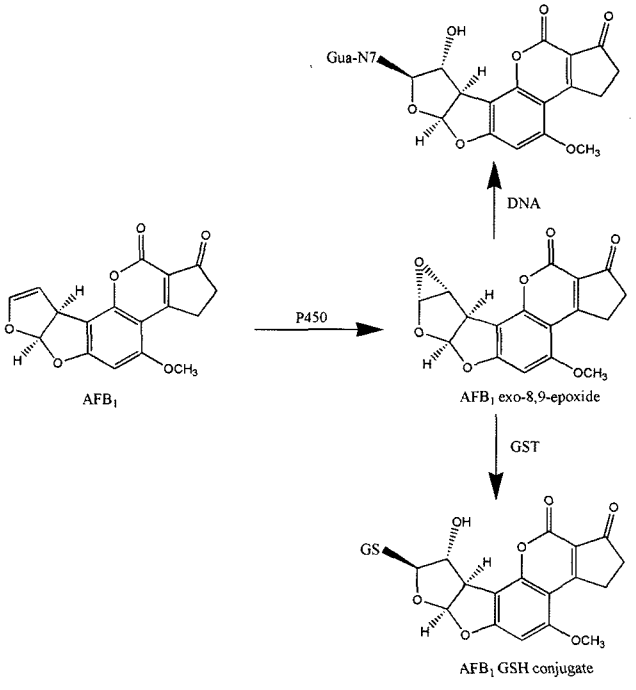


**Fig. 4.** Major events in the metabolism of $AFB_1$.

The interaction of $AFB_1$ *exo*-8,9-epoxide with DNA involves the intercalation of the epoxide between base pairs, a peripheral proton field that appears to facilitate both hydrolysis and conjugation, and a very facile $S_N2$ reaction of the guanyl $N^7$ atom that is probably imposed by intercalation and proximity effects (Raney *et al.*, 1990; Johnson and Guengerich, 1997; Guengerich *et al.*, 1998) (Fig. 4).

The *exo*-8,9-epoxide can be enzymatically conjugated with glutathione (GSH) by GSH transferase M1 (Fig. 4). Epidemiology studies have given conflicting results to date on the role of the GSH transferase M1 polymorphism in AFB1-related liver cancer (Guengerich, 2001). The hydrolysis product (dihydrodiol) is unstable and undergoes base-catalyzed rearrangement to a reactive dialdehyde, which appears to be a major species involved in the reaction with proteins (but not DNA) (Guengerich, 2006).

***Trichloroethylene (TCE).*** 1,1,2-Tricholoethylene (TCE) is a volatile organic solvent that is widely used as a degreasing agent, a polymer precursor, and a dry cleaning agent. It has become a major environmental pollutant and is one of the most abundant organic contaminants found in water dump sites (Gist and Burg, 1995). TCE was metabolized via two general pathways, an oxidative pathway in which the first step is catalyzed by P450 or a GSH-dependent pathway in which the initials step was catalyzed by GSH transferase (Cai and Guengerich, 2001). P450 oxidation of TCE yields $Cl_3CCHO$ [chloral (hydrate)] and TCE oxide (a minor product) (Fig. 5). TCE oxide has a very short half life (< 12 s) at neutral pH but it can react with protein lysines via conjugation. The analysis of TCE reaction with the oligopeptide adrenocorticotrophic hormone of the pituitary gland (ACTH) showed an example of the covalent binding of a reactive electrophile with a protein (Guengerich, 2006).

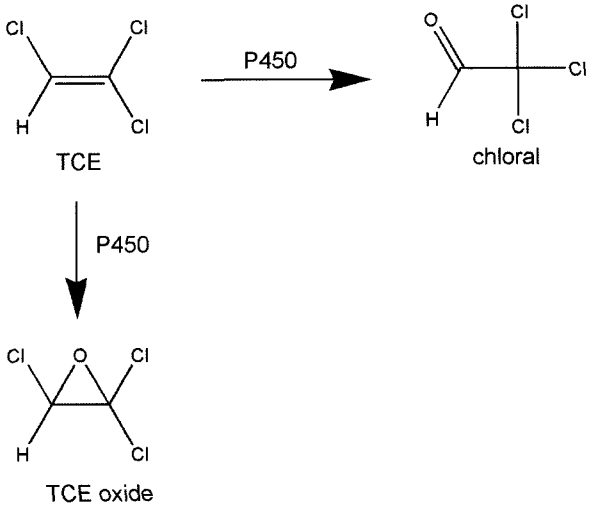


**Fig. 5.** Oxidation of TCE by P450s.

## GENOTOXICITY ASSAY SYSTEMS INCLUDING HUMAN P450

Exposure to toxic chemicals causes many human cancers via DNA damage. Bruce Ames has devised a simple and sensitive test for detecting chemical mutagens using *Salmonella* stain (Ames *et al.*, 1975). HAAs have long been known to be mutagenic following metabolic activation and showed a strong mutagenicity in *S. typhimurium* strains (Nebert *et al.*, 1979). HAAs preferentially induce the frameshift mutations in CG repeat of the *his*$D^+$ gene, as opposed to cause base pair mutations (Fuscoe *et al.*, 1988). This kind of mutation hotspot is also found in other bacterial genes such as the *lacZ*, *lacZa*, and *lacI* of *Escherichia coli* (Kosakarn *et al.*, 1993; Watanabe and Ohta, 1993). An *E. coli lacZ* reversion mutation assay was applied to study HAA genotoxicity (Josephy, 2000). An *E. coli* tester stain carrying a (-GC) copy of *lacZ* gene can regain functional *lacZ* activity following induction of frameshifts by HAAs (Marwood *et al.*, 1995). Systems have also been developed that incorporate the heterologous expression of P450s and NADPH-P450 reductase (Josephy *et al.*, 1998). This system allows the detection of HAA mutagenicity by recombinant human P450 without a need for rat liver fractions. These bacteria have also been genetically engineered to express *S. typhimurium* NAT, and the DNA nucleotide excision repair system has been inactivated (UvrABC) in order to improve the sensitivity (Watanabe *et al.*, 1987). These *E. coli* strains overexpressing P450s and NAT have been used to characterize CYP1A2 allelic and random variants (Parikh *et al.*, 1999; Kim and Guengerich, 2004). Another use of this genotoxicity system was to screen and characterize P450 inhibitors. For instance, a CYP1B1-based system was used to characterize the potent inhibition of the enzyme by tetramethylstilbene and a CYP1A2-based system was sensitive to the drug oltipraz (Chun *et al.*, 2001; Langouët *et al.*, 2000). Other bacterial systems have utilized the SOS response in *S. typhimurium* NM2009 to measure DNA damage (Oda *et al.*, 1995). This strain contains a plasmid-based *umuDC* gene linked to a *lacZ* reporter gene and was activated by induction of the SOS pathway (Oda *et al.*, 1995). Sensitivity to arylamines and HAAs was also improved by incorporating plasmids coding for P450 enzymes, NADPH-P450 reductase, and NAT (Oda *et al.*, 2001).

## CONCLUDING REMARKS

Estimating toxicity is an important topic in the human health-related chemicals including carcinogens, environmental pollutants, and drugs. Many chemicals are inert unless converted to the activated metabolites by P450s or other enzymes. Reactive electrophiles produced in this way are clearly important in toxicity, as demonstrated by several examples in this review. In addition, it should be kept in mind that human cancer risk associated with P450-activated chemicals depends on the level of dietary exposure in the population, the biologically effective doses arising from those exposures within relevant target tissues, and the relationship between these effective doses and predicted increased cancer risk. It is very difficult to estimate the risk of P450-activated chemicals to humans, because of the differences of polymorphisms in metabolic enzymes. The exact role of metabolic polymorphisms in the risks of individuals and their epidemiological studies must be considered in evaluations of human toxicity of P450-activated chemicals.

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