Mol. Nutr. Food Res. 2005, *49*, 101 – 117 DOI 10.1002/mnfr.200400076

Review

Interspecies metabolism of heterocyclic aromatic amines and the uncertainties in extrapolation of animal toxicity data for human risk assessment

Robert J. Turesky

National Center for Toxicological Research, Division of Chemistry, Jefferson, AR, USA

Heterocyclic aromatic amines (HAAs) are potent bacterial mutagens that are formed in cooked meats, tobacco smoke condensate, and diesel exhaust. Many HAAs are carcinogenic in experimental animal models. Because of their wide-spread occurrence in the diet and environment, HAAs may contribute to some common types of human cancers. The extrapolation of animal toxicity data on HAAs to assess human health risk has many uncertainties, which can lead to tenuous risk assessment estimates. Perhaps the most critical and variable parameters in interspecies extrapolation are the effects of dose, species differences in catalytic activities of xenobiotic metabolism enzymes (XMEs), human XME polymorphisms that lead to interindividual differences in carcinogen metabolism, and dietary constituents that may either augment or diminish the carcinogenic potency of these genotoxins. The impact of these parameters on the metabolism and toxicological properties of HAAs and uncertainties in extrapolation of animal toxicity data for human risk assessment are presented in this article.

Keywords: Cancer risk / Heterocyclic aromatic amines / Interspecies metabolism / Polymorphisms / Review

Received: July 30, 2004; revised: October 18, 2004; accepted: October 18, 2004

Contents

Correspondence: Dr. Robert J. Turesky, Division of Environmental Disease and Prevention, Wadsworth Center, New York State Department of Health, P.O. Box 509, Albany, NY 12201-0509, USA
E-mail: Rxt07@health.state.ny.us
Fax: +1-518-486-1505

Abbreviations: AA, aromatic amine; **2-AαC**, 2-amino-9*H*-pyrido[2,3-*b*]indole; **CYP**, cytochrome P450; **7,9-DiMeIgQx**, 2-amino-1,7,9-trimethylimidazo[4,5-*g*]quinoxaline; **4,8-DiMeIQx**, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline; **7,8-DiMeIQx**, 2-amino-3,7,8-trimethylimidazo[4,5-*f*]quinoxaline; **GSH**, glutathione; **GST**, glutathione *S*-transferase; **HAA**, heterocyclic aromatic amine; **IQ**, 2-amino-3-methylimidazo[4,5-*f*]quinoline; **IQx**, 2-amino-3-methylimidazo[4,5-*f*]quinoxaline; **MeAαC**, 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole; **8-MeIQx**, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; **NAT**, *N*-acetyltransferase; **PhIP**, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]-pyridine; **SULT**, sulfotransferase; **UGT**, UDP-glucuronosyltransferase; **XME**, xenobiotic metabolism enzyme

1 Introduction

More than 20 heterocyclic aromatic amines (HAAs) are formed in meat, fish, and poultry prepared under common household cooking practices [1, 2]. A number of HAAs have also been detected in tobacco smoke condensate, beer, wine, and diesel fuel exhaust [2 – 6], which can lead to contamination of the water supply [7]. Two classes of HAAs have been identified. One class is formed through the condensation of creatinine with fragmented hexoses and pyrazine or pyridine derivatives that occur during cooking of foods to form aminoimidazoarenes or the 2-amino-3-

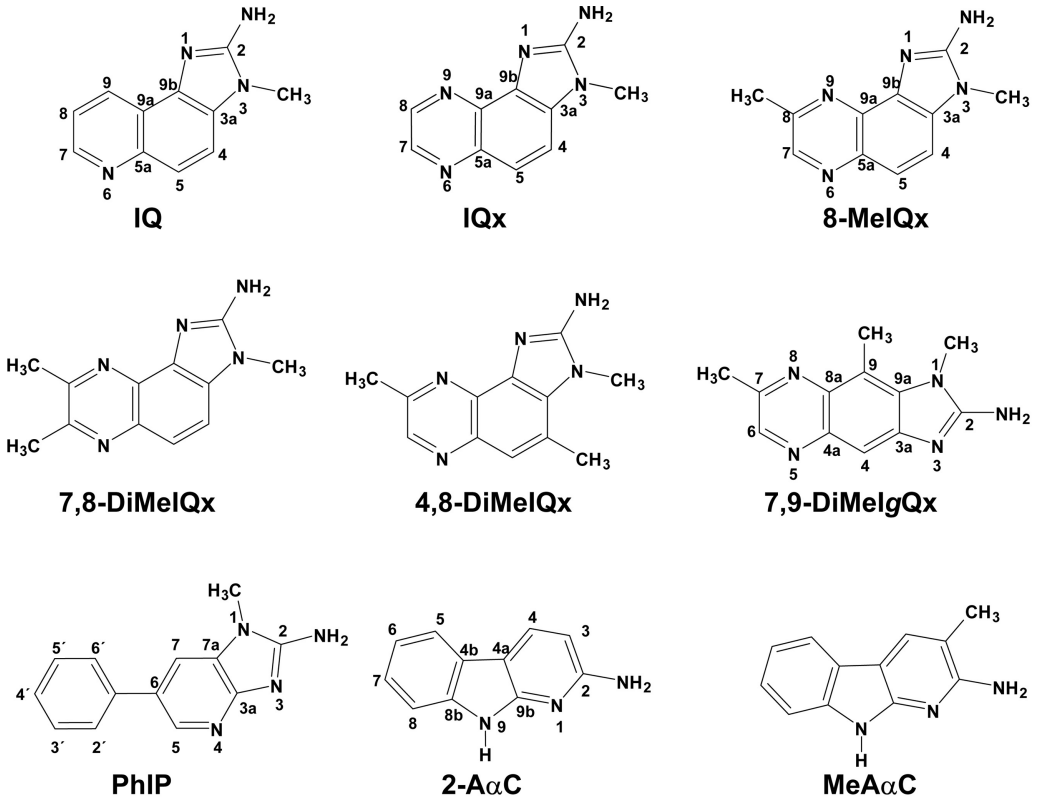

Figure 1. Chemical structures of some of the more prevalent HAAs formed in cooked meats. More than 20 HAAs have been identified in cooked meats [1, 2].

methylimidazo[4,5-*f*]quinoline (IQ)- and 2-amino-3-methylimidazo[4,5-*f*]quinoxaline (IQx)-type compounds [3]. A second class of HAAs, the pyrolysis-type HAAs, are formed through thermal decomposition of amino acids, such as tryptophan or proteins at elevated temperature [1, 8, 9]. The concentration of HAAs in cooked meats usually occurs at the low parts per billion (ppb) level; however, the concentration of HAAs may exceed 300 ppb in some well-done grilled or barbecued meats [10, 11]. The chemical structures of some of the more prevalent HAAs are shown in Fig. 1.

HAAs are potent bacterial mutagens that induce tumors at multiple sites in experimental laboratory animals [1]. One HAA, namely IQ, is also a powerful liver carcinogen in non-human primates [12]. Because of the wide-spread occurrence of HAAs in cooked meats, it is thought that HAAs may contribute to the etiology of several common human cancers that are often associated with frequent meat consumption [1, 13 – 17].

Many genotoxins, including HAAs, require metabolism to exert their biological effects. The extrapolation of animal carcinogenicity data to assess human health risk of HAAs requires consideration of species differences in the catalytic activities and expression of xenobiotic metabolism enzymes (XMEs) that are involved in the bioactivation and detoxication of HAAs [18]. In contrast to many experimental animal models, humans show large interindividual variation in the levels of expression of phase I and II XMEs. Many of the XMEs involved in HAA metabolism in humans display common genetic polymorphisms that may affect the levels of protein expression, protein stability and catalytic activities, which influence the biotransformation of toxicants and health risk in exposed populations [19 – 23]. These species differences in the levels of XME expression and catalytic acitivities may lead to uncertainties in extrapolation of animal toxicity data for human risk assessment of HAAs and are highlighted in this review.

2 HAA metabolism by phase I and II enzymes

The metabolic pathways of the several HAAs formed in cooked meats, IQ, 2-amino-3,8-dimethylimidazo[4,5-*f*]-quinoxaline (8-MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), and 2-amino-9*H*-pyrido[2,3-*b*]-indole (2-AαC), that have been reported in experimental animals and humans, are depicted in Fig. 2. Both phase I and II enzymes that include cytochrome P450s (CYPs), sulfotrans-

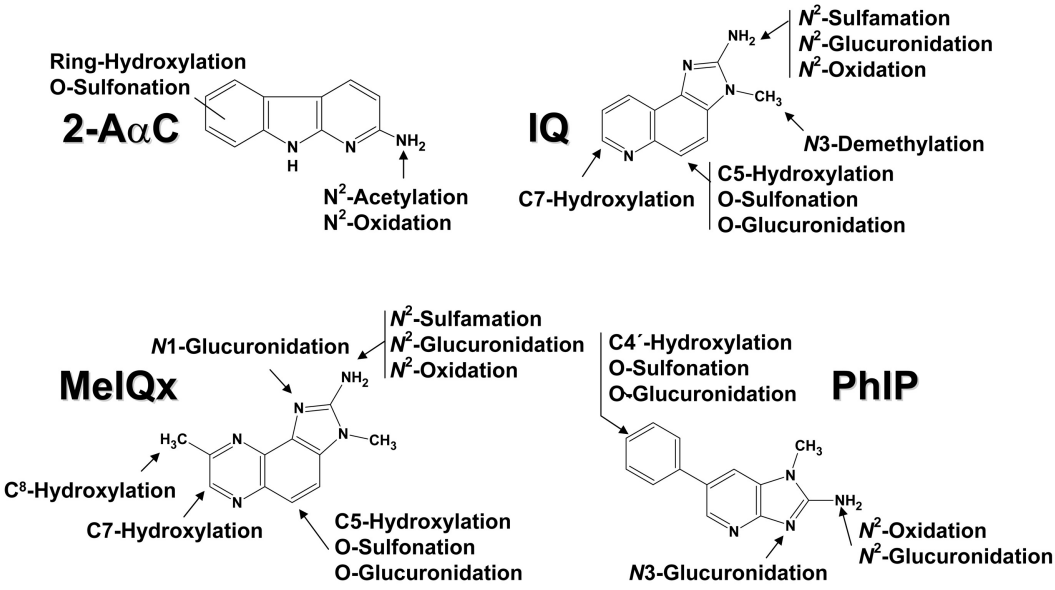

Figure 2. Major pathways of HAA metabolism in experimental animal models and humans.

ferases (SULTs), UDP-glucuronosyltransferases (UGTs), *N*-acetyltransferases, and glutathione *S*-transferases (GSTs), are involved in HAA metabolism [24 – 27]. CYPs catalyze the oxidation at the aromatic ring system of HAAs to produce detoxicated products and also oxidize the exocyclic amine groups of HAAs to produce the genotoxic *N*-hydroxy-HAA metabolites. The *N*-hydroxy-HAA metabolites have a number of biochemical fates as shown in Fig. 3. The *N*-hydroxy-HAAs may bind to DNA, or undergo further activation through phase II enzymes, such as NATs or SULTs to form unstable esters that readily react with DNA principally at the C8 and N^2 atoms of deoxyguanosine [28 – 30]. Alternatively, some of the electrophilic *N*-hydroxy-HAAs and esters of *N*-hydroxy-HAAs may undergo reduction back to the parent amines by glutathione (GSH) alone, or through catalysis with GSTs, possibly through the formation of unstable GSH conjugates as intermediates [31, 32]. An NADPH-dependent reductase [33, 34] also has been reported to reduce several *N*-hydroxy-HAAs back to the parent amines as a mechanism of detoxication. Peroxidases and prostaglandin H synthases (COX) also may contribute to the bioactivation of HAAs by oxidation of the exocyclic amine groups [16, 35, 36]. The peroxidases may be of particular importance in breast tissue, a target of some HAAs in experimental animal models and possibly in humans [16, 35, 37, 38]. For IQ and MeIQx, direct conjugation of the exocyclic amine group occurs through SULTs leading to stable sulfamic acid derivatives. These sulfamates are important detoxication products in rodents, nonhuman primates, and humans [24, 39 – 43]. UGT-catalzyed glucuronidation of HAAs and *N*-hydroxy-HAA metabolites at the excocyclic or imidazole nitrogen atoms is another important metabolic pathway that occurs in various animal species and in humans [24, 26, 27, 41, 44 – 46].

3 Cytochrome P450 metabolism

Based upon *in vitro* metabolism studies, liver microsomal fractions containing CYPs are the most active tissue in oxidation of HAAs [25, 47]. CYP1A2 (for nomenclature see: www.imm.ki.se/CYPalleles/), which is principally expressed in liver [18, 48, 49], is the predominant CYP involved in the *N*-oxidation of carcinogenic aromatic amines (AAs) and HAAs in rodents and humans [25, 47, 50 – 53]. Both CYP 1A1 and 1B1 have been reported to catalyze this *N*-oxidation pathway of HAAs in extrahepatic tissues [54 – 56].

3.1 Species differences in regioselectivity of CYP1A2-catalyzed metabolism of HAAs

The human and rat CYP1A2 are 75% identical in amino acid sequence [57, 58]. Despite these similarities, the changes in amino acid sequence may have a profound effect on the enzyme substrate specificity and catalytic activity. The regioselectivity of HAA oxidation by human and rat CYP1A2 is quite different. Human CYP1A2 is regioselective for the *N*-oxidation of HAAs, such as IQ, MeIQx, and PhIP (bioactivation), and does not catalyze the oxidation (detoxication) of the heteroaromatic ring systems [42, 53, 59]. Human CYP1A2 also catalyzes the three-step oxidation of the C^8-methyl group of 8-MeIQx to form the car-

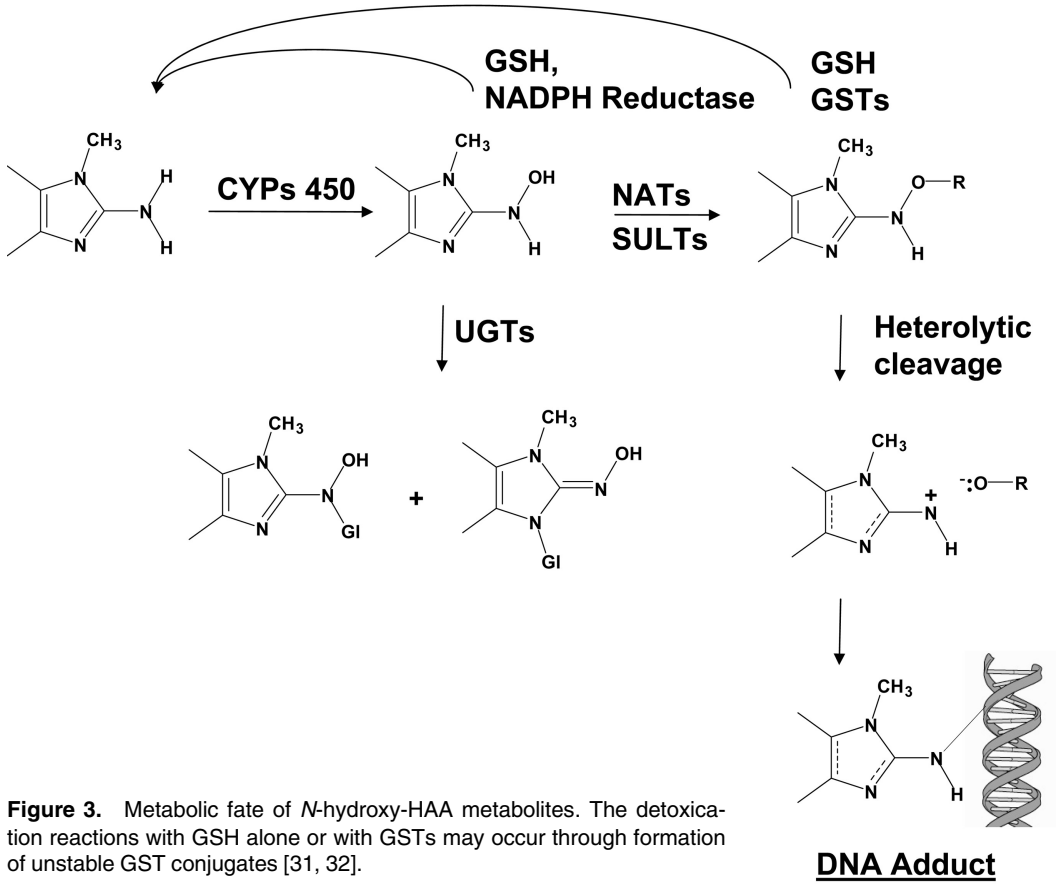

Figure 3. Metabolic fate of *N*-hydroxy-HAA metabolites. The detoxication reactions with GSH alone or with GSTs may occur through formation of unstable GST conjugates [31, 32].

boxylic acid derivative, 2-amino-3-methylimidazo[4,5-*f*]-quinoxaline-8-carboxylic acid [27, 60], a major pathway of detoxication 8-MeIQx *in vivo* [42]. This oxidation pathway is specific for human CYP1A2, and CYPs of rodents and nonhuman primates do not form this product [24, 41]. The 4′-hydroxylation of PhIP has been reported to occur in human hepatocytes [61], and the oxidation product is excreted in urine of humans predominantly as the sulfate conjugate [45]. However, 4′-HO-PhIP formation is not catalyzed by CYP1A2, nor do other CYPs in human liver appear to catalyze this oxidation process [27, 53, 61]. In contrast to human CYP1A2, the rodent isoform does catalyze the ring oxidation of IQ, MeIQx, and PhIP, and these are major pathways of metabolism and detoxication of these HAAs in rodents [24, 26, 53, 62]. These heterocyclic ring oxidation products are also observed nonhuman primates that have been used for studies on metabolism and long-term carcinogen biossays of HAAs [63]. However, CYP1A2 was reported not to be expressed in liver of this species (Cynomolgus monkeys) [64] and other CYPs, including CYP3A4 and/or CYP2C9/10 appear to contribute to both ring and exocyclic *N*-oxidation of HAAs [63].

In contrast to the aminoimidazoarene HAAs, the pyrolysis-derived HAAs, 2-AαC and MeAαC, undergo extensive ring oxidation by human CYP1A2 and other CYPs [65, 66]. Thus, important species differences in the regioselectivity of CYP-catalyzed oxidation of HAAs exist, which may influence the toxicological properties of these procarcinogens.

3.2 Differences in kinetic parameters of rat and human CYP1A2 in *N*-oxidation of HAAs and ensuing DNA damage

The kinetic parameters of HAA *N*-oxidation by purified rat CYP1A2 and recombinant human CYP1A2 are different. The catalytic efficiency (k_{cat}/K_m) of recombinant human CYP1A2 in *N*-oxidation of MeIQx and PhIP was reported to be 10- and 19-fold greater than that of CYP1A2 of the rat [53, 67], a species used in long-term carcinogen bioassays (see Fig. 4) [1, 68]. In the case of MeIQx, the K_m values for rat CYP1A2- and recombinant human CYP1A2-mediated *N*-oxidation of MeIQx were similar (Fig. 4) but the k_{cat} for human CYP1A2 was 16-fold greater than that of rat CYP1A2. Comparable k_{cat} activities were observed for rat and recombinant human CYP1A2-catalyzed *N*-oxidation of PhIP; however, the K_m value for human CYP1A2 was 13-fold lower than the K_m of rat CYP1A2, resulting in a 19-fold

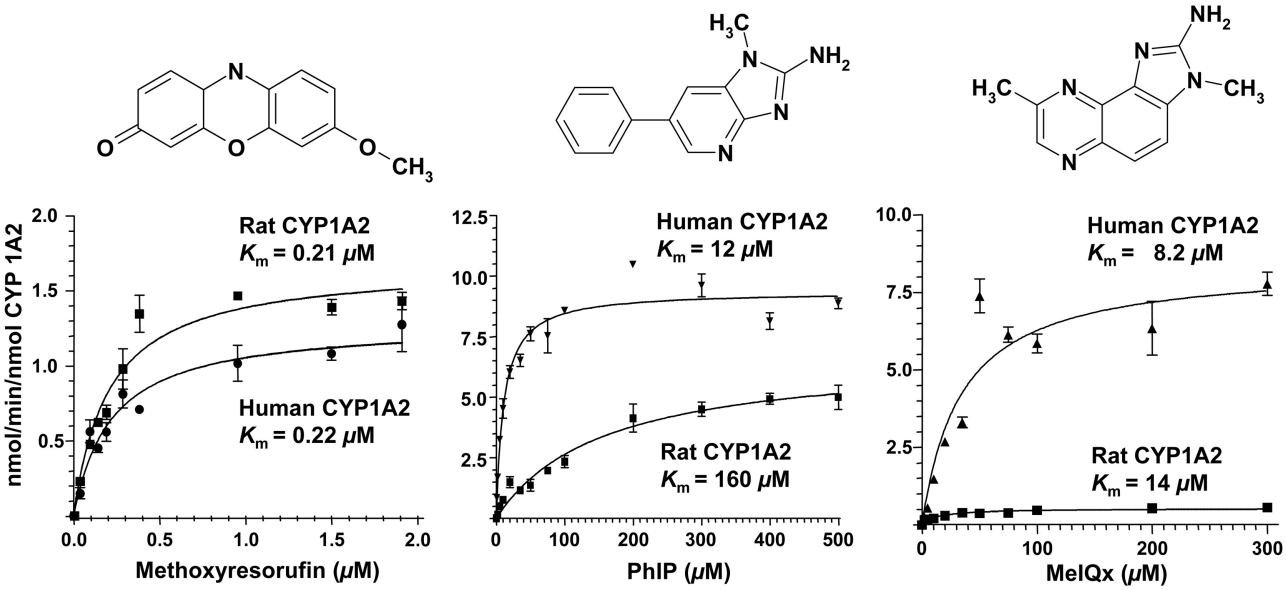

Figure 4. Kinetic parameters of MeIQx and PhIP *N*-oxidation and methoxyresorufin *O*-demethylation by purified rat CYP1A2 and recombinant human CYP1A2. Adapted from [67].

greater catalytic efficiency (k_{cat}/K_m). These differences in catalytic activities were also observed in human and rat liver microsomes [53]. However, the k_{cat} and K_m values determined for methoxyresorufin *O*-demethylation with purified rat CYP1A2 and recombinant human CYP1A2 were comparable [53]. Thus, there are important differences in catalytic activities of rat and human CYP1A2, which depend upon the chemical structure of the substrate.

Several studies have shown that some human liver S-9 fractions or microsomal samples are highly active at transforming grilled meat extracts containing HAAs and HAAs into bacterial mutagens [53, 67, 69 – 71]. The abilities of human and rat liver microsomal samples to transform MeIQx and PhIP into bacterial mutagens were investigated in the Ames reversion assay using *Salmonella typhimurium* TA98 strain (Fig. 5). Many human liver samples were more efficient than rat liver samples at activating these HAAs to mutagens. In fact, a number of human liver microsomal samples were as effective at inducing mutants as liver microsomal samples of rats pretreated with polychlorinated biphenyls, a powerful inducer of CYP1A2 and CYP1A1 [47, 50, 72], which also catalyzes the *N*-oxidation of MeIQx and PhIP [25, 53, 62, 73]. The higher mutagenicity induced by many human liver microsomal samples compared to untreated rat liver microsomal samples is consistent with the superior catalytic activity of human CYP1A2 towards *N*-oxidation of HAAs, and the greater level of CYP1A2 expression in human liver than liver of untreated rodents [53].

Differences in CYP1A2 activities also exist between human and rat hepatocytes on *N*-oxidation of HAAs, where the percent of the dose of MeIQx and PhIP transformed into *N*-glucuronide conjugates of these *N*-hydroxy-HAAs is greater in human than rat hepatocytes [27]. These findings are consistent with superior catalytic activity displayed with human CYP1A2 than rat CYP1A2 in microsomal assays and with the purified proteins [53]. Human CYP1A2 is a major enzyme involved in the biotransformation of MeIQx and PhIP in human hepatocytes based upon studies with furafylline, a selective, mechanism-based inhibitor of CYP1A2 [74]. Pretreatment of hepatocytes with furafylline resulted in a strong, concentration-dependent decrease in the amounts of the *N*-glucuronide conjugates of *N*-hydroxy-MeIQx and *N*-hydroxy-PhIP, and the 8-carboxylic acid oxidation product of MeIQx by more than 90%, while the amounts of unmetabolized PhIP and MeIQx increased by more than 10-fold [27].

The importance of CYP1A2 in metabolism of MeIQx and PhIP in humans *in vivo* has been demonstrated through pharmacokinetic studies with furafylline, where as much as 91% of the elimination of MeIQx and 70% of the PhIP could be accounted for by CYP1A2-catalyzed metabolism [75]. In the case of MeIQx, the 8-carboxylic acid derivative, which is formed by CYP1A2 [60], accounts for more than 50% of the metabolites of MeIQx in human urine [42]. Moreover, a higher percent of the dose of MeIQx and PhIP also appears to be converted into *N*-hydroxylated metabolites in humans than rodents *in vivo*, based upon the detection of the *N*-glucuronide conjugates of the *N*-hydroxy-HAA metabolites in urine [24, 42, 45, 46]. The superior catalytic efficiency of human CYP1A2 over rat CYP1A2 in

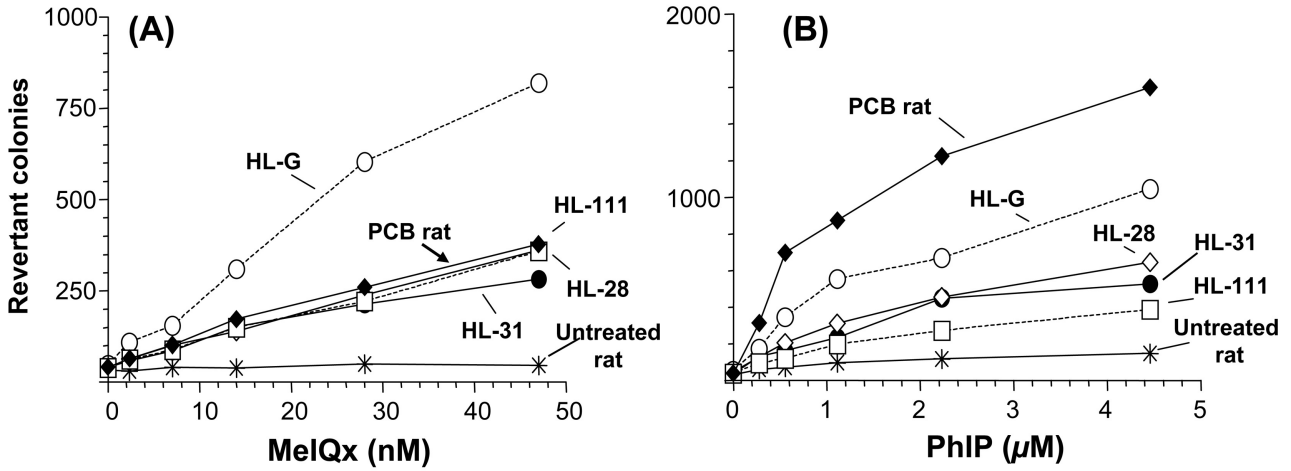

Figure 5. Mutagenicity of (A) MeIQx and (B) PhIP in the Ames reversion assay with four different human liver microsomal samples with high (HL-G) and intermediate levels (HL-28, HL-31, and HL-111) of CYP1A2 expression and rat liver microsomes obtained from an untreated rat and a rat pretreated with polychlorinated biphenyls. All assays were done with microsomal protein (20 μg/mL). Adapted from [53].

N-oxidation of HAAs also appears to correlate to a greater percentage of the HAAs that is converted to the biologically effective dose *in vivo* that binds to DNA. A direct comparison of the abilities of rats and human subjects to form HAA-DNA adducts *in vivo* was assessed by accelerator mass spectrometry (AMS), a highly sensitive technique to measure radiolabeled isotopes at trace levels [76]. AMS analysis revealed that a higher percentage of the dose of MeIQx bound to colon DNA of humans than colon DNA of rats or mice [77 – 79]. In another study, PhIP-DNA adducts were detected in human breast [80]. These data demonstate that HAA-DNA adduct formation can occur in humans at low, dietary relevant concentrations, and that humans appear to be more efficient than rodents at biotransformation of HAAs to the genotoxic *N*-hydroxy metabolites both *in vitro* and *in vivo*.

3.3 Human CYP1A2 polymorphisms affecting HAA metabolism

In addition to species differences in catalytic activities of CYP1A2, there are also important species differences in the levels of expression of CYP1A2. The constitutive CYP1A2 mRNA expression levels in human liver can vary by as much as 15-fold [81, 82], and interindividual expression of CYP1A2 protein may vary by 60-fold in human liver [48, 49, 53, 83]. In contast to humans, the level of expression of CYP1A2 protein in liver of several different strains of rat varies by only several fold [53]. The variable expression of CYP1A2 in humans is attributed to environmental and dietary factors, such as smoking [84 – 86], consumption of cruciferous vegetables [87, 88], and grilled meats [89], which induce CYP1A2 expression. Several genetic polymorphisms [90, 91] and a variation in the level of CpG methylation [82] have been detected in the upstream 5′-regulatory region of the *CYP1A2* gene that affect the level of *CYP1A2* mRNA expression and may lead to variations in the level of protein expression. An example of the interindividual variation in CYP1A2 protein expression in human liver samples (5–250 pmol/mg microsomal protein, median 71 pmol/mg, $N = 51$) is shown in Fig. 6. Forty-three out of the 51 human liver samples contain higher CYP1A2 protein levels than liver samples of rats, where CYP1A2 content ranged from 5 to 35 pmol/mg microsomal protein, depending upon the strain, source, and diet [53]. The wide range in human CYP1A2 levels is paralleled by a large variation in the rates of *N*-oxidation of MeIQx and PhIP, which correlate to the levels of CYP1A2. The rates of *N*-oxidation of MeIQx and PhIP are much lower in rat liver microsomes, which is reflective of the lower amounts of CYP1A2 protein expressed in rat liver and the poorer catalytic activity of rat CYP1A2 relative to human CYP1A2 (Figs. 4 and 6) [53]. The rates of *N*-oxidation and ranges of interindividual variation of CYP1A2 activity towards 4-aminobiphenyl, 4,4′-methylenebis-2-chloroaniline [51, 92], IQ [51, 92], and 2-AαC [65] by human liver microsomes are similar to those values cited for MeIQx and PhIP.

There are at least ten different allelic variants in the coding region of the *CYP1A2* gene [93, 94]. Several of these allelic variants display altered catalytic activities towards several HAAs [95] and other CYP1A2 substrates [94]. In individuals with high CYP1A2 activity, the amount of unmetabolized MeIQx recovered in urine was lower than in individuals with low CYP1A2 activity, suggesting that CYP1A2 is an important enzyme in the metabolism of MeIQx *in*

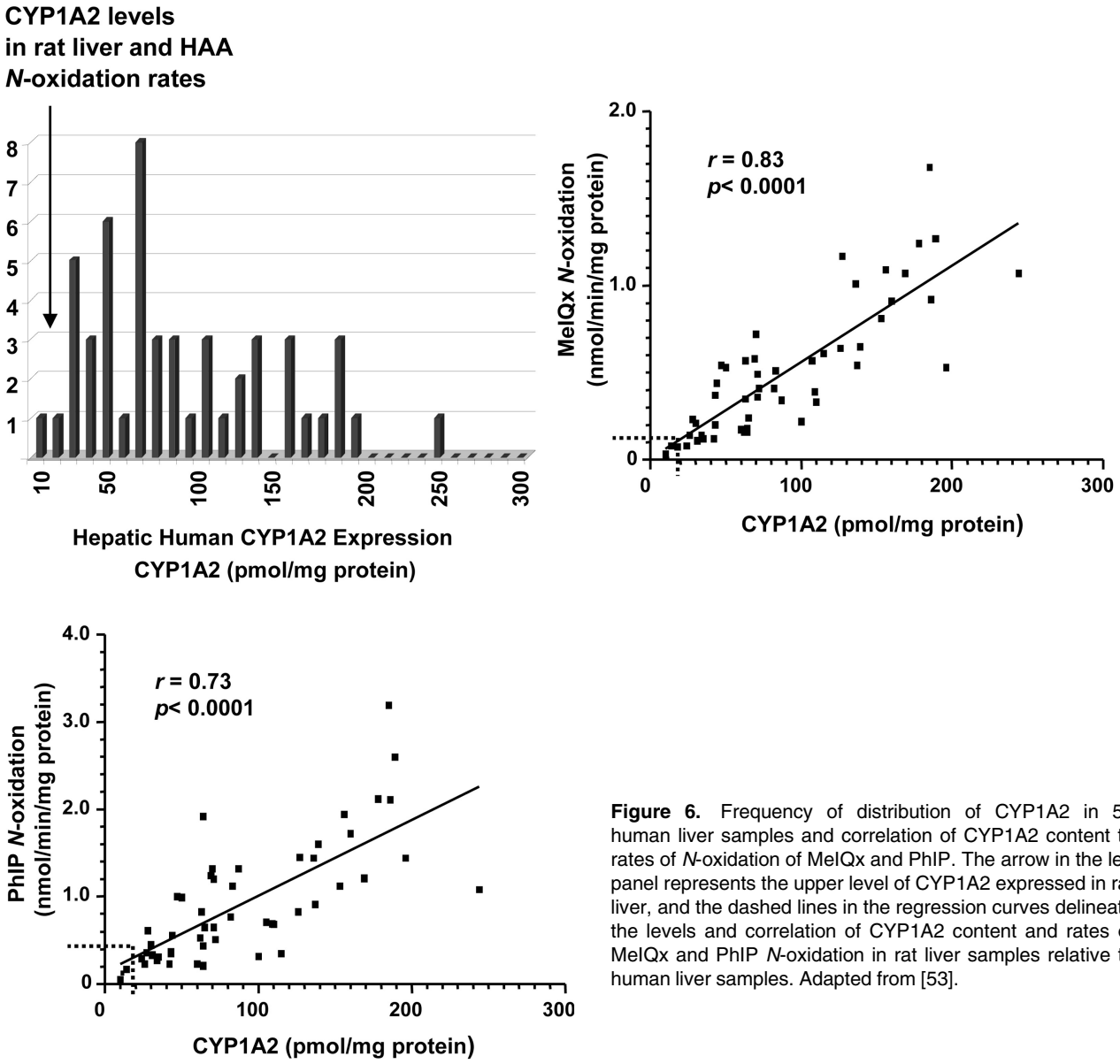

Figure 6. Frequency of distribution of CYP1A2 in 51 human liver samples and correlation of CYP1A2 content to rates of *N*-oxidation of MeIQx and PhIP. The arrow in the left panel represents the upper level of CYP1A2 expressed in rat liver, and the dashed lines in the regression curves delineate the levels and correlation of CYP1A2 content and rates of MeIQx and PhIP *N*-oxidation in rat liver samples relative to human liver samples. Adapted from [53].

vivo. In contrast to MeIQx, a poor correlation was observed between CYP1A2 activity and the levels of unmetabolized PhIP in urine of the same individuals, suggesting that CYP1A2 may be less important in PhIP metabolism in vivo. However, GSTs have been reported to catalyze the reduction of CYP1A2-mediated *N*-hydroxylated metabolites of PhIP back to the parent compound, and this GST-mediated detoxication pathway of *N*-hydroxy-PhIP may have confounded the correlation between CYP1A2 activity and PhIP metabolism. The prevalence of CYP1A2 polymorphisms in human populations and their possible effects on HAA metabolism and DNA adduct formation requires investigation.

4 Metabolism of *N*-hydroxy-HAAs by phase II enzymes

The genotoxic effects of the *N*-hydroxy-HAAs are greatly influenced by phase II enzymes, such as *N*-acetyltransferases (NATs) (for nomenclature see: www.louisville.edu/medschool/pharmacology/NAT.html) and sulfotransferases (SULTs) (for nomenclature see: [96]), which catalyze the binding of *N*-hydroxy-HAA metabolites to DNA through formation of highly reactive esters that undergo heterolytic cleavage to produce the reactive nitrenium ion [29, 30] (Fig. 2). Phosphorylases and aminoacyl-tRNA synthetases also have been reported to activate *N*-hydroxy-HAAs in

experimental animals, although the contribution of these phase II enzymes to bioactivation of *N*-hydroxy-HAAs in humans has not been studied extensively [30]. A number of genetic polymorphisms in phase II enzymes have been reported and may impact the toxicological properties of HAAs [97 – 100].

4.1 *N*-Acetyltransferases and polymorphisms affecting HAA metabolism

There are at least two and ten polymorphic genotypes of human NAT1 and NAT2, respectively [19, 20, 99]. NAT1 is primarily expressed in extrahepatic tissues, while NAT2 is expressed predominantly in the liver and intestinal epithelium [101, 102]. NATs metabolize a number of clinical drugs containing amine and hydrazine functional groups [20]. The *N*-acetylation of AAs is recognized as a major detoxication pathway in arylamine metabolism in experimental animals and humans [19, 103]. In addition, NATs catalyze the intramolecular *N,O*-acetyltransfer of *N*-hydroxy-*N*-acetyl-AAs to produce reactive *N*-acetoxy intermediates that bind to DNA [19, 103]. It is noteworthy that many HAAs are poor substrates for NAT and do not undergo *N*-acetylation as a mechanism of detoxication in either rodents or humans [28, 104, 105]. Instead, the NATs catalyze bioactivation of the *N*-hydroxy-HAAs to form the highly reactive *N*-acetoxy esters that readily bind to DNA [106]. The *N*-acetoxy derivatives of *N*-hydroxy-IQ and *N*-hydroxy-MeIQx are highly unstable with lifetimes of seconds or less [29]. In contrast to these HAAs, *N*-acetoxy-PhIP is sufficiently stable and has been isolated by HPLC [107]. The *O*-acetylation of many *N*-hydroxy-HAAs is catalyzed to a greater extent by human NAT2 than NAT1 [106], although *N*-hydroxy-PhIP and *N*-hydroxy-2-AαC, in particular, are bioactivated by NAT1 at appreciable levels [106, 108]. Both rapid and slow *O*-acetylator phenotypes of *N*-hydroxy-HAAs are present in human liver samples [28]. *O*-Acetylation of *N*-hydroxy-HAAs also occurs in human colon, a putative target tissue of HAAs; however, a rapid *O*-acetylation polymorphism is not clearly discerned in colon [28], which may be due to the relatively low expression of NAT2 protein in colon [101]. The bioactivation of *N*-hydroxy-HAAs may be carried out largely by NAT1 in this tissue. The NAT1 enzyme also contributes to the bioactivation of several *N*-hydroxy-HAAs in human breast, where NAT2 activity is low [109]. The NAT1 is expressed at relatively high levels in extrahepatic tissues [101, 102] and may contribute to bioactivation of *N*-hydroxy-HAAs in putative target tissues such as the colon, prostate and mammary glands [110 – 112]. PhIP has been identified in breast milk of healthy women who ate well-done meat [113], and a recent study reported the detection of PhIP-DNA adducts in human breast tissue by means of immunohistochemistry [114]. Moreover, among well-done meat consumers, women with a rapid *NAT2* genotype were reported to have a higher level of PhIP-DNA adducts than those individuals with a slow *NAT2* genotype. This finding suggests that *N*-acetoxy-PhIP formed by NAT2 in liver may be sufficiently stable to be transported through the blood stream and react with DNA in the breast. Further investigations on the potential role of PhIP and other HAAs in breast cancer are warranted.

The NAT2 fast acetylator type (*NAT2*4* wild-type allele) has been linked with increase risk of colorectal cancer in some epidemiological studies [19, 20]. Moreover, a positive interactive effect was observed among individuals who frequently consumed grilled meats and who were both rapid acetylators and CYP1A2 *N*-oxidizers, which suggests a putative role for HAAs in the etiology of colorectal cancer [115 – 117]. However, the molecular epidemiology data on the associations between drug metabolism activity (CYP1A2 or NAT2), consumption of cooked meat, and colorectal cancer development are inconsistent, and the findings of some studies have questioned the role of HAAs and these associations in colorectal cancer risk [20, 91, 118, 119] (and references within). The elevated risk of colorectal cancer may be conferred only in individuals who are both rapid NAT2 and CYP1A2 phenotypes and who are frequent consumers of cooked meat products containing high concentrations of HAAs [115, 116].

4.2 Sulfotransferases and polymorphisms affecting HAA metabolism

The human SULTs also catalyze the sulfation of *N*-hydroxy-HAAs that may bind to DNA and enhance mutagenicity, particularly, for the *N*-hydroxy derivatives of PhIP, 2-AαC, and MeAαC [108, 120 – 122]. However, *N*-hydroxy-IQ and *N*-hydroxy-MeIQx do not appear to be substrates for human SULTs and DNA binding is not enhanced in the presence of these enzymes [121, 123]. The highest SULT activities for *N*-hydroxy-HAAs are associated with SULT1A1, followed by SULT1A2 and SULT1A3, and SULT1B1 [120, 122]. SULT1E1, which is under hormonal regulation and inducible by progesterone, also was reported to enhance the binding of *N*-hydroxy-PhIP to DNA in cultured human epithelial cells [124]. Depending upon the *N*-hydroxy-HAA substrate, *N*-sulfation catalyzed DNA binding by some human liver cytosols is greater than liver cytosols of rats or mice [108, 120]. SULTs are expressed in a wide variety of tissues, including breast and colon [110, 111, 113], putative target tissues of PhIP and other HAAs in humans [1, 77]. In addition to bioactivation, SULTs also may contribute to detoxication of HAAs. Sulfamation is an important detoxication pathway of IQ and MeIQx (Fig. 2) in rodents, nonhuman primates, and humans [24, 42]. Human SULT1A1 appears to be the principal isoform involved in this conjugation pathway [43]. The sulfamation of PhIP is also catalyzed by rat liver

cytosols [120]; however, this pathway has not been reported *in vivo* in rodents or humans [24, 26, 45, 46].

A large interindividual variation has been observed in bioactivation of *N*-hydroxy-HAAs by SULTs in human liver cytosols [123]. Several common polymorphisms have been reported for SULT1A1, which may affect the expression or stability of the protein and catalytic activity [125 – 127]. One common polymorphism in SULT1A1 occurs at codon 213, where a G → A transition results in an arginine (*SULT1A1*1* (wild type)) to histidine substitution (*SULT1A1*2*) [127, 128]. Subjects with the homozygous *His* allele display substantial decreases in *N*-sulfation catalyzed DNA binding of *N*-hydroxy-4-aminobiphenyl and *N*-hydroxy-PhIP [97]. It is noteworthy that one recent epidemiological study [129] reported that women who were frequent consumers of well-done meat and who carried the *His*/*His* genotype with diminished *N*-sulfation activity of *N*-hydroxy-HAAs and *N*-hydroxy-AAs, were at decreased risk for breast cancer relative to women who carried the functional homozygous wild type *Arg*/*Arg* allele (*SULT1A1*1*). These findings are suggestive of a role of AAs or HAAs as etiological agents in breast cancer. The detection of PhIP-DNA adducts in breast tissue by immunochemical detection leads credence to the potential role of this carcinogen in breast cancer development [114]. Further investigations on the roles of HAAs and *SULT* polymorphisms in breast cancer are warranted.

4.3 UDP-glucuronosyltransferases and polymorphisms affecting HAA metabolism

The UGT-catalyzed glucuronidation of many HAAs and their *N*-hydroxy-HAA metabolites occurs at the excocyclic or imidazole nitrogen atoms to form stable conjugates. Glucuronidation is a major metabolic pathway of detoxication of these dietary carcinogens in rodents, nonhuman primates, and prominently in humans [24, 26, 27, 41, 42, 44 – 46]. The conjugation is catalyzed by a number of UGT1A isoforms (for nomenclature see: http://som.flinders.edu.au/FUSA/ClinPharm/UGT/currnom.htm) that are expressed in human liver and extrahepatic tissues [100, 130]. The highest activities were observed for UGT1A1, UGT1A9, followed by UGT1A8, UGT1A7, and UGT 1A3, which catalyzed the N^2 and N^3 glucuronidation of *N*-hydroxy-PhIP but with different regioselectivities [131 – 133]. The rates of glucuronidation of *N*-hydroxy-PhIP by some human colon samples approached the levels observed in human liver, demonstrating the importance of UGT-catalyzed detoxication of this carcinogenic metabolite in colon, a putative target organ of PhIP-induced carcinogenesis [131]. The UGT isoforms that contribute to the metabolism of other HAAs, such as IQ and MeIQx, have not been reported but most likely also involve the 1A family [24]. Several common genetic polymorphisms have been reported in the UGT1A family [100, 130]. Since significant interindividual variation of glucuronidation of MeIQx, PhIP, as well as their *N*-hydroxylated metabolites has been reported in humans [11, 42, 46, 134], the the role of *UGT* polymorphisms in metabolism and detoxication of HAAs may be an important factor to consider in the risk assessment of these genotoxins.

4.4 Glutathione *S*-transferases and polymorphisms affecting HAA metabolism

The GSTs (for nomenclature see [135, 136]) are another important class of phase II enzymes involved in the detoxication of numerous xenobiotics that include: endogenous electrophiles, aldehydes and radicals derived from lipid peroxidation products, and various genotoxins, including *N*-acetoxy-PhIP [135, 137]. The family of cytosolic GSTs and a second family of structurally unrelated insoluble microsomal GSTs have been identified but their capacity to detoxicate HAAs have not been reported. There are eight classes of cytosolic GSTs (Alpha, Kappa, Mu, Omega, Pi, Sigma, Theta, and Zeta). The catalytically active cytosolic proteins are dimers of subunits from within the same class of proteins [135, 137]. Amongst the *N*-hydroxy-HAAs, only *N*-acetoxy-PhIP has been reported to be a substrate for GSTs, which diminished the binding of this reactive PhIP metabolite to DNA [31, 98]. Human GST A1-1 (α-class) was reported to most efficiently detoxicate this carcinogen, followed by A2-2, P1-1, T1-1, T2-2, which displayed lower activities, while no activities were detected for GSTs A4-4, M2-2, M4-4, and Z1-1 [31, 98]. Analyses of incubation mixtures containing *N*-acetoxy-PhIP, glutathione (GSH) and GST A1-1 revealed the presence of oxidized GSH (GSSG) and PhIP but not GSH conjugates of PhIP [31]. The stoichiometry of GSSG and PhIP formation suggested that the reaction was not a simple redox reaction; however, the formation of an unstable GSH conjugate of PhIP as an intermediate can not be excluded. The DNA-binding of *N*-acetoxy-IQ, *N*-acetoxy-MeIQx, and *N*-acetoxy-2-AαC were not diminished by GSTs, but some of the respective *N*-hydroxy- and *N*-acetoxy-HAAs underwent partial reduction back to the parent amines by GSH alone [31, 32, 108].

The expression and distribution of GSTs is highly variable in humans and inducible [138, 139], suggesting the potential for chemoprevention strategies towards *N*-acetoxy-PhIP. The highest level of expression of the GST α-class occurs in the liver, while the amount of GSTA1 expressed in colon is approximately 100-fold less [140 – 142]. A common polymorphism was reported in the 5′-regulatory region of *GSTA1*. The *GSTA1*B* variant was associated with 4-fold lower mean hepatic GSTA1 expression compared to *GSTA1*A* [143]. In a case-control study for colorectal cancer, approximately 35% of a Caucasian population contained

**A/*A* genotype, followed by 51% **A/*B* heterozygotes, and 14% **B/*B* homozygotes in the control population, while the **B/*B* individuals were over represented in cases at 24% [98, 143]. The odds ratio for risk of colorectal cancer with homozygous **B/*B* individuals was 2.0. However, when the analysis was conducted with preference for well-done meat, which contain high concentrations of PhIP and other HAAs [2], the health risk association was apparent only with frequent consumption of well-done beef where the odds ratio of **B/*B* individuals was approximately 3-fold relative to the **A/*A* and **A/*B* genotypes [32]. These protective effects of GSTA1 observed in individuals who frequently consume well-done meat are suggestive of an etiologic role of PhIP or other HAAs in colorectal cancer.

A mechanistic model for the chemical carcinogenesis of colorectal cancer has been proposed based upon the metabolism of PhIP and other HAAs in cooked meats [32, 144]. The bioactivation of PhIP occurs in the liver through CYP1A2-catalyzed *N*-oxidation, followed by NAT-catalyzed *O*-acetylation of *N*-hydroxy-PhIP to form *N*-acetoxy-PhIP [32, 144]. The *N*-acetoxy-PhIP intermediate is readily inactivated by GSTA1 in the liver of individuals of **A/*A* and **A/*B* genotypes; however, relatively higher amounts of *N*-acetoxy-PhIP may escape inactivation in the liver of **B/*B* individuals who express less GSTA1 and higher amounts of this genotoxin are transported into the blood stream. In the colon, a presumed target organ of PhIP, the protective effects of GSTs are largely lacking due to the poor expression of GSTA1, and *N*-acetoxy-PhIP may readily form DNA adducts leading to mutations [32]. In this mechanistic model, the protective effects of GSTA1 would only be detected in individuals who are exposed to HAAs and who display both rapid CYP1A2 and NAT activities to form the critical genotoxic PhIP metabolite [32]. However, other XMEs expressed in liver or colon that are involved in detoxication of *N*-hydroxy-PhIP, such as UGTs [131, 132, 145], may provide significant protection against this genotoxin and may obscure the interaction amongst frequent meat consumption, *GSTA1* genotypes, and colorectal cancer risk. In an independent small pilot study on colorectal cancer patients ($N = 76$), PhIP-DNA adducts were approximately 2-fold higher in the lymphocytes of **B/*B* carriers than in **A/*A* and **A/*B* genotypes [146]. This data reinforces the notion that *GSTA1* polymorphisms and exposure to PhIP may be important risk factors for colorectal cancer development.

4.5 Challenges of in the extrapolation of interspecies toxicity data to assess human health risk of HAAs

The cancer risk of HAAs is uncertain and risk factor estimates have ranged widely. An upper limit was estimated at 1 cancer case per 1000 individuals [147], intermediate risk factors of 1 in 5000, and 1 in 10 000 [148–152], and a lower limit of 50 cancer cases per 10^6 individuals was reported [153]. These divergent estimates can be attributed to the different assumptions that are used in calculating risk factors. The estimated daily intake of HAAs, which may vary by more than 100-fold in cooked meats [10, 11, 154], can lead to disparate risk factor estimates. Another important parameter is the usage of different total doses for 50% tumor incidence values (TD_{50}) from various animal carcinogen bioassays, which show strong differences in HAA carcinogenic potencies [1, 147, 152]. Dose concentrations used in animal carcinogen bioassays are another important variable, where the doses of HAAs have been conducted at 10^4- to 10^6-fold greater amounts than daily human exposure [1, 68]. These high levels of HAA exposure may result in metabolic pathways that preferentially lead to formation of chemically reactive metabolites, which may not arise under low-dose treatments. High-dose exposure may also lead to saturation of enzymatic detoxication systems and result in an enhanced HAA toxicity. However, an approximate linear response in IQ-DNA [155] and MeIQx-DNA [76, 79] adduct formation has been observed in rats as a function of dose over a wide range of dose treatments, indicating that a constant proportion of these HAAs are bioactivated to genotoxic species. Tumor incidence in animal models and extrapolation of DNA adduct-based measurements for human cancer risk assessment have been evaluated for several different classes of carcinogens [156–159]. However, HAA-DNA adduct formation occurs at significant levels in many tissues of experimental animal models that do not develop cancer [30], indicating that factors in addition to DNA adduct formation, such as base misincorporation during translesional synthesis, cell proliferation, and other tumor promotion factors are required for cancer development [68, 160–162].

Another variable between species which may affect the toxicity of HAAs is the diet. In contrast to the standardized diet of experimental animals, the human diet is highly diverse and complex, and there are numerous dietary constituents that may obscure the extrapolation of animal toxicity data to assess human health risk. For example, there are components in grilled meats [89] that increase the expression of CYP1A2 [89], which may increase the bioactivation of HAAs in humans. Caffeine, tea, and coffee also have been reported to increase the expression of hepatic CYP1A2 in rats [163–166], and caffeine has been reported to modulate the genotoxic potential of HAAs *in vivo* [167]. Fortunately, dietary components, such as cruciferous vegetables containing indole-3-carbinol and organic isothiocyanate derivatives [88, 168–170] and beverages, such as coffee and tea, contain constituents that inhibit CYP1A2-catalyzed *N*-oxidation of HAAs [171, 172]. Constituents in these foods and beverages also increase the expression of

phase II enzymes that detoxicate HAAs [87, 138, 172 – 174]. Conjugated linoleic acids also have been reported to modulate the metabolism of several HAAs through a decrease in CYP-catalyzed *N*-oxidation activity, which results in decreased levels of DNA adduct formation and a decreased incidence of tumor incidence in experimental animal models [175, 176]. Two extensive review articles have recently been published on dietary constituents that may influence the toxicological properties and health risks associated with HAAs [177, 178].

Another uncertainty in risk assessment of HAAs is the bioavailability of HAAs in cooked foods, where the estimates of HAAs in cooked meats may not be reflective of the actual daily exposure levels and, thus, affect risk estimates. For example, the bioavailability of HAAs may be reduced by dietary constituents, such as chlorophyll, which bind to HAAs [179], or by the cooked meat matrix, where increasing the doneness of the meat appears to decrease the amount of HAA accessible from the meat matrix [180]. Moreover, cooked meats contain a variety of carcinogens at low concentrations that include: polycyclic aromatic hydrocarbons, *N*-nitroso compounds, lipid peroxides, and other pro-oxidative agents, and fungal products, in addition to HAAs. The carcinogenic potency of grilled meats and health risk may be related not only to HAAs, but to this complex mixture of genotoxic compounds [1, 181 – 185], all of which are affected by various XME polymorphisms. Because of these complex genetic-environment interactions, it may be difficult to identify a polymorphism of a specific XME and correlate it to the heath risk associated with one class of genotoxins in cooked meats.

5 Conclusions

The biochemical data summarized in this review highlights some of the many important species differences in XMEs that both activate and detoxicate HAAs. Comparison between experimental animals and human CYPs and phase II enzymes is essential for the extrapolation of animal carcinogenicity data to assess human health risk, and consideration of species differences in catalytic activities of these enzymes is important. The low level of CYP1A2 expressed in rat liver combined with the strong differences between rat and human CYP1A2 activities in HAA activation suggest that the carcinogenicity data of the rat may underestimate the human health risk of HAAs. However, interspecies comparisons of HAA toxicity must be made with caution as XME detoxication pathways may be different in humans and experimental animals and affect the toxicological properties of HAAs. Moreover, the large interindividual variations in the expression of CY1A2 and phase II enzymes that are involved in both the bioactivation and detoxication of HAAs most likely results in different health risks for individuals. The development of sensitive analytical mass spectrometry methods has enabled investigators to measure HAAs [134, 186, 187], their metabolites [42, 46] and DNA adducts in humans [77, 78, 188]. These chemical markers may be used to more accurately determine the inter-relationship amongst dietary factors, XME polymorphisms, and susceptibility factors in HAA diet-related carcinogenesis [23, 189, 190].

Disclaimer: The views presented in this article do not necessarily reflect those of the United States Food and Drug Administration.

6 References

[1] Sugimura, T., Wakabayashi, K., Nakagama, H., Nagao, M., Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci.* 2004, *95*, 290 – 299.

[2] Felton, J. S., Jagerstad, M., Knize, M. G., Skog, K., Wakabayashi, K., Contents in Foods, Beverages and Tobacco. In: Nagao, M., Sugimura, T. (Eds.), *Food Borne Carcinogens Heterocyclic Amines*, John Wiley & Sons, Chichester, England 2000, pp. 31 – 71.

[3] Skog, K. I., Johansson, M. A., Jägerstad, M. I., Carcinogenic heterocyclic amines in model systems and cooked foods: a review on formation, occurrence and intake. *Food Chem. Toxicol.* 1998, *36*, 879 – 896.

[4] Manabe, S., Suzuki, H., Wada, O., Ueki, A., Detection of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in beer and wine. *Carcinogenesis* 1993, *14*, 899 – 901.

[5] Manabe, S., Izumikawa, S., Asakuno, K., Wada, O., Kanai, Y., Detection of carcinogenic amino-α-carbolines and amino-γ-carbolines in diesel-exhaust particles. *Environ. Pollut.* 1991, *70*, 255 – 265.

[6] Kanai, Y., Wada, O., Manabe, S., Detection of carcinogenic glutamic acid pyrolysis products in cigarette smoke condensate. *Carcinogenesis* 1990, *11*, 1001 – 1003.

[7] Kataoka, H., Hayatsu, T., Hietsch, G., Steinkellner, H., *et al.*, Identification of mutagenic heterocyclic amines (IQ, Trp-P-1 and AlphaC) in the water of the Danube river. *Mutat. Res.* 2000, *466*, 27 – 35.

[8] Sugimura, T., Nagao, N., Kawachi, T., Honda, M., *et al.*, Mutagen-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. In: Hiatt, H. H., Watson, J. D., Winstein, J. A. (Eds.), *Origins of Human Cancer, Book C*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY 1977, pp. 1561 – 1577.

[9] Matsumoto, T., Yoshida, D., Tomita, H., Determination of mutagens, amino-α-carbolines in grilled foods and cigarette smoke condensate. *Cancer Lett.* 1981, *12*, 105 – 110.

[10] Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P., *et al.*, High concentrations of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) occur in chicken but are dependent on the cooking method. *Cancer Res.* 1995, *55*, 4516 – 4519.

[11] Felton, J. S., Knize, M. G., Salmon, C. P., Malfatti, M. A., Kulp, K. S., Human exposure to heterocyclic amine food mutagens/carcinogens: relevance to breast cancer. *Environ. Mol. Mutagen.* 2002, *39*, 112 – 118.

[12] Adamson, R. H., Thorgeirsson, U. P., Snyderwine, E. G., Reeves, J., *et al.*, Carcinogenicity of 2-amino-3-methylimidazo[4,5-*f*]quinoline in nonhuman primates: induction of tumors in three macaques. *Jpn. J. Cancer Res.* 1990, *81*, 10 – 14.

[13] Nagao, M., Sugimura, T., Carcinogenic factors in food with relevance to colon cancer development. *Mutat. Res.* 1993, *290*, 43 – 51.

[14] Shirai, T., Sano, M., Tamano, S., Takahashi, S., *et al.*, The prostate: A target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) derived from cooked foods. *Cancer Res.* 1997, *57*, 195 – 198.

[15] Snyderwine, E. G., Some perspectives on the nutritional aspects of breast cancer research. Food-derived heterocyclic amines as etiologic agents in human mammary cancer. *Cancer* 1994, *74*, 1070 – 1077.

[16] DeBruin, L. S., Josephy, P. D., Perspectives on the chemical etiology of breast cancer. *Environ. Health Perspect.* 2002, *110 Suppl 1*, 119 – 128.

[17] Zheng, W., Gustafson, D. R., Sinha, R., Cerhan, J. R., *et al.*, Well-done meat intake and the risk of breast cancer. *J. Natl. Cancer Inst.* 1998, *90*, 1724 – 1729.

[18] Guengerich, F. P., Comparisons of catalytic selectivity of cytochrome P450 subfamily members from different species. *Chem.-Biol. Interact.* 1997, *106*, 161 – 182.

[19] Hein, D. W., *N*-Acetyltransferase genetics and their role in predisposition to aromatic and heterocyclic amine-induced carcinogenesis. *Toxicol. Lett.* 2000, *112 – 113*, 349 – 356.

[20] Hein, D. W., Doll, M. A., Fretland, A. J., Leff, M. A., *et al.*, Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol. Biomarkers Prev.* 2000, *9*, 29 – 42.

[21] Pavanello, S., Clonfero, E., Biological indicators of genotoxic risk and metabolic polymorphisms. *Mutat. Res.* 2000, *463*, 285 – 308.

[22] Evans, W. E., Relling, M. V., Pharmacogenomics: translating functional genomics into rational therapeutics. *Science* 1999, *286*, 487 – 491.

[23] Kelada, S. N., Eaton, D. L., Wang, S. S., Rothman, N. R., Khoury, M. J., The role of genetic polymorphisms in environmental health. *Environ. Health Perspect.* 2003, *111*, 1055 – 1064.

[24] King, R. S., Kadlubar, F. F., Turesky, R. J., *In vivo* metabolism of heterocyclic amines. In: Nagao, M., Sugimura, T. (Eds.), *Food Borne Carcinogens: Heterocyclic Amines*, John Wiley & Sons, Chichester, England 2000, pp. 90 – 111.

[25] Yamazoe, Y., Nagata, K., *In vitro* metabolism. In: Sugimura, T., Nagao, M. (Eds.), *Food Borne Carcinogens Heterocyclic Amines*. John Wiley & Sons, Chichester, England 2000, pp. 74 – 89.

[26] Alexander, J., Heidenreich, B., Reistad, R., Holme, J. A., Metabolism of the food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in the rat and other rodents. In: Adamson, R. H., Gustafsson, J.-A., Ito, N., Nagao, M., Sugimura, T., Wakabayashi, K., Yamazoe, Y. (Eds.), *Heterocyclic amines in cooked foods: Possible human carcinogens. 23rd Proceedings of the Princess Takamatusu Cancer Society*, Princeton Scientific Publishing, Princeton, NJ 1995, pp. 59 – 68.

[27] Turesky, R. J., Guengerich, F. P., Guillouzo, A., Langouet, S., Metabolism of heterocyclic aromatic amines by human hepatocytes and cytochrome P4501A2. *Mutat. Res.* 2002, *506 – 507*, 187 – 195.

[28] Turesky, R. J., Lang, N. P., Butler, M. A., Teitel, C. H., Kadlubar, F. F., Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. *Carcinogenesis* 1991, *12*, 1839 – 1845.

[29] Turesky, R. J., DNA adducts of heterocyclic aromatic amines, arylazides and 4-nitroquinoline 1-oxide. In: Hemminki, K., Dipple, A., Shuker, D. E. G., Kadlubar, F. F., Segerbäck, D., Bartsch, H. (Eds.), *DNA Adducts: Identification and Biological Significance*, International Agency for Research on Cancer, Lyon 1994, pp. 217 – 228.

[30] Schut, H. A., Snyderwine, E. G., DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. *Carcinogenesis* 1999, *20*, 353 – 368.

[31] Lin, D.-X., Meyer, D. J., Ketterer, B., Lang, N. P., Kadlubar, F. F., Effects of human and rat glutathione-*S*-transferase on the covalent binding of the *N*-acetoxy derivatives of heterocyclic amine carcinogens *in vitro*, a possible mechanism of organ specificity in their carcinogenesis. *Cancer Res.* 1994, *54*, 4920 – 4926.

[32] Coles, B. F., Kadlubar, F. F., Detoxification of electrophilic compounds by glutathione *S*-transferase catalysis: Determinants of individual response to chemical carcinogens and chemotherapeutic drugs? *Biofactors* 2003, *17*, 115 – 130.

[33] King, R. S., Teitel, C. H., Shaddock, J. G., Casciano, D. A., Kadlubar, F. F., Detoxification of carcinogenic aromatic and heterocyclic amines by enzymatic reduction of the *N*-hydroxy derivative. *Cancer Lett.* 1999, *143*, 167 – 171.

[34] Kim, D., Kadlubar, F. F., Teitel, C. H., Guengerich, F. P., Formation and reduction of aryl and heterocyclic nitroso compounds and significance in the flux of hydroxylamines. *Chem. Res. Toxicol.* 2004, *17*, 529 – 536.

[35] Williams, J. A., Stone, E. M., Millar, B. C., Hewer, A., Phillips, D. H., Pathways of heterocyclic amine activation in the breast: DNA adducts of 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) formed by peroxidases and in human mammary epithelial cells and fibroblasts. *Mutagenesis* 2000, *15*, 149 – 154.

[36] Wiese, F. W., Thompson, P. A., Kadlubar, F. F., Carcinogen substrate specificity of human COX-1 and COX-2. *Carcinogenesis* 2001, *22*, 5 – 10.

[37] Josephy, P. D., The role of peroxidase-catalyzed activation of aromatic amines in breast cancer. *Mutagenesis* 1996, *11*, 3 – 7.

[38] Snyderwine, E. G., Mammary Gland. In: Nagao, M., Sugimura, T. (Eds.), *Food Borne Carcinogens Heteroyclic Aromatic Amines*, John Wiley & Sons, Chichester 2000, pp. 241 – 255.

[39] Turesky, R. J., Skipper, P. L., Tannenbaum, S. R., Coles, B., Ketterer, B., Sulfamate formation is a major route for detoxification of 2-amino-3-methylimidazo[4,5-*f*]quinoline in the rat. *Carcinogenesis* 1986, *7*, 1483 – 1485.

[40] Sjödin, P., Wallin, H., Alexander, J. A., Jägerstad, M., Disposition and metabolism of the food mutagen 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in rats. *Carcinogenesis* 1989, *10*, 1269 – 1275.

[41] Snyderwine, E. G., Turesky, R. J., Turteltaub, K. W., Davis, C. D., *et al.*, Metabolism of food-derived heterocyclic amines in nonhuman primates. *Mutat. Res.* 1997, *376*, 203 – 210.

[42] Turesky, R. J., Garner, R. C., Welti, D. H., Richoz, J., *et al.*, Metabolism of the food-borne mutagen 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in humans. *Chem. Res. Toxicol.* 1998, *11*, 217 – 225.

[43] Ozawa, S., Nagata, K., Yamazoe, Y., Kato, R., Formation of 2-amino-3-methylimidazo[4,5-*f*]quinoline- and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline-sulfamates by cDNA-expressed mammalian phenol sulfotransferases. *Jpn. J. Cancer Res.* 1995, *86*, 264 – 269.

[44] Styczynski, P. B., Blackmon, R. C., Groopman, J. D., Kensler, T. W., The direct glucuronidation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) by human and rabbit liver microsomes. *Chem. Res. Toxicol.* 1993, *6*, 846 – 851.

[45] Kulp, K. S., Knize, M. G., Malfatti, M. A., Salmon, C. P., Felton, J. S., Identification of urine metabolites of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine following consumption of a single cooked chicken meal in humans. *Carcinogenesis* 2000, *21*, 2065 – 2072.

[46] Kulp, K. S., Knize, M. G., Fowler, N. D., Salmon, C. P., Felton, J. S., PhIP metabolites in human urine after consumption of well-cooked chicken. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* 2004, *802*, 143 – 153.

[47] Kato, R., Yamazoe, Y., Metabolic activation and covalent binding to nucleic acids of carcinogenic heterocyclic amines from cooked foods and amino acid pyrolysates. *Jpn. J. Cancer Res.* 1987, *78*, 297 – 311.

[48] Shimada, T., Yamazaki, H., Mimura, M., Inui, Y., Guengerich, F. P., 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.* 1994, *270*, 414 – 423.

[49] Eaton, D. L., Gallagher, E. P., Bammler, T. K., Kunze, K. L., Role of cytochrome P450 1A2 in chemical carcinogenesis: implications for human variability in expression and enzyme activity. *Pharmacogenetics* 1995, *5*, 259 – 274.

[50] Kamataki, T., Maeda, K., Yamazoe, Y., Matsuda, N., *et al.*, A high-spin form of cytochrome P-450 highly purified from polychlorinated biphenyl-treated rats. *Mol. Pharmacol.* 1983, *24*, 146 – 155.

[51] Butler, M. A., Iwasaki, M., Guengerich, F. P., Kadlubar, F. F., Human cytochrome P-450$_{PA}$ (P450IA2), the phenacetin *O*-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and *N*-oxidation of carcinogenic arylamines. *Proc. Natl. Acad. Sci. USA* 1989, *86*, 7696 – 7700.

[52] Rich, K. J., Murray, B. P., Lewis, I., Rendell, N. B., *et al.*, *N*-Hydroxy-MeIQx is the major microsomal oxidation product of the dietary carcinogen MeIQx with human liver. *Carcinogenesis* 1992, *13*, 2221 – 2226.

[53] Turesky, R. J., Constable, A., Richoz, J., Varga, N., *et al.*, Activation of heterocyclic aromatic amines by rat and human liver microsomes and by purified rat and human cytochrome P450 1A2. *Chem. Res. Toxicol.* 1998, *11*, 925 – 936.

[54] Shimada, T., Yun, C.-H., Yamazaki, H., Gautier, J.-C., *et al.*, Characterization of human lung microsomal cytochrome P-450 1A1 and its role in the oxidation of chemical carcinogens. *Mol. Pharmacol.* 1992, *41*, 856 – 864.

[55] Crofts, F. G., Sutter, T. R., Strickland, P. T., Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by human cytochrome P4501A1, P4501A2 and P4501B1. *Carcinogenesis* 1998, *19*, 1969 – 1973.

[56] Shimada, T., Hayes, C. L., Yamazaki, H., Amin, S., *et al.*, Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res.* 1996, *56*, 2979 – 2984.

[57] Jaiswal, A. K., Nebert, D. W., McBride, O. W., Gonzalez, F. J., Human P_3-P450: cDNA and complete protein sequence, repetitive *Alu* sequences in the 3′ nontranslated region and localization of gene to chromosome 15. *J. Exp. Pathol.* 1987, *3*, 1 – 17.

[58] Ikeya, K., Jaiswal, A. K., Owens, R. A., Jones, J. E., *et al.*, Human CYP1A2: sequence, gene structure, comparison with the mouse and rat orthologous gene, and differences in liver 1A2 mRNA expression. *Mol. Endocrinol.* 1989, *3*, 1399 – 1408.

[59] Hammons, G. J., Milton, D., Stepps, K., Guengerich, F. P., Kadlubar, F. F., Metabolism of carcinogenic heterocyclic and aromatic amines by recombinant human cytochrome P450 enzymes. *Carcinogenesis* 1997, *18*, 851 – 854.

[60] Turesky, R. J., Parisod, V., Huynh-Ba, T., Langouët, S., Guengerich, F. P., Regioselective differences in C(8)- and *N*-oxidation of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline by human and rat liver microsomes and cytochromes P450 1A2. *Chem. Res. Toxicol.* 2001, *14*, 901 – 911.

[61] Langouët, S., Paehler, A., Welti, D. H., Kerriguy, N., *et al.*, Differential metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in rat and human hepatocytes. *Carcinogenesis* 2002, *23*, 115 – 122.

[62] Wallin, H., Mikalsen, A., Guengerich, F. P., Ingelman-Sundberg, I., *et al.*, Differential rates of metabolic activation and detoxification of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by different cytochrome P450 enzymes. *Carcinogenesis* 1990, *11*, 489 – 492.

[63] Snyderwine, E. G., Turesky, R. J., Turteltaub, K. W., Davis, C. D., *et al.*, Metabolism of food-derived heterocyclic amines in nonhuman primates. *Mutat. Res.* 1997, *376*, 203 – 210.

[64] Edwards, R. J., Murray, B. P., Murray, S., Schulz, T., *et al.*, Contribution of CYP1A1 and CYP1A2 to the activation of heterocyclic amines in monkeys and humans. *Carcinogenesis* 1994, *15*, 829 – 836.

[65] Raza, H., King, R. S., Squires, R. B., Guengerich, F. P., *et al.*, Metabolism of 2-amino-α-carboline. A food-borne heterocyclic amine mutagen and carcinogen by human and rodent liver microsomes and by human cytochrome P450 1A2. *Drug Metab. Disp.* 1996, *24*, 395 – 400.

[66] Frederiksen, H., Frandsen, H., Impact of five cytochrome p450 enzymes on the metabolism of two heterocyclic aromatic amines, 2-amino-9*H*-pyrido[2,3-*b*]indole (AalphaC) and 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAalphaC). *Pharmacol. Toxicol.* 2003, *92*, 246 – 248.

[67] Turesky, R. J., Constable, A., Fay, L. B., Guengerich, F. P., Interspecies differences in metabolism of heterocyclic aromatic amines by rat and human P450 1A2. *Cancer Lett.* 1999, *143*, 109 – 112.

[68] Sugimura, T., Overview of carcinogenic heterocyclic amines. *Mutat. Res.* 1997, *376*, 211 – 219.

[69] Shimada, T., Iwasaki, M., Martin, M. V., Guengerich, F. P., Human liver microsomal cytochrome P-450 enzymes involved in the bioactivation of procarcinogens detected by *umu* gene response in *Salmonella typhimurium* TA 1535/pSK1002. *Cancer Res.* 1989, *49*, 3218 – 3228.

[70] Felton, J. S., Healy, S. K., Activation of mutagens in cooked ground beef by human liver microsomes. *Mutat. Res.* 1984, *140*, 61 – 65.

[71] Hakura, A., Suzuki, S., Sawada, S., Sugihara, T., *et al.*, Use of human liver S9 in the Ames test: assay of three procarcinogens using human S9 derived from multiple donors. *Regul. Toxicol. Pharmacol.* 2003, *37*, 20 – 27.

[72] Yamazoe, Y., Abu-Zeid, M., Manabe, S., Toyama, S., Kato, R., Metabolic activation of a protein pyrolysate promutagen 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline by rat liver microsomes and purified cytochrome P-450. *Carcinogenesis* 1988, *9*, 105 – 109.

[73] Holme, J. A., Wallin, H., Brunborg, G., Soderlund, E. J., *et al.*, Genotoxicity of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP): formation of 2-hydroxyamino-PhIP, a directly acting genotoxic metabolite. *Carcinogenesis* 1989, *10*, 1389 – 1396.

[74] Kunze, K. L., Trager, W. F., Isoform-selective mechanism-based inhibition of human cytochrome P450 1A2 by furafylline. *Chem. Res. Toxicol.* 1993, *6*, 649 – 656.

[75] Boobis, A. R., Lynch, A. M., Murray, S., de la Torre, R., *et al.*, CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. *Cancer Res.* 1994, *54*, 89 – 94.

[76] Turteltaub, K. W., Felton, J. S., Gledhill, B. L., Vogel, J. S., *et al.*, Accelerator mass spectrometry in biomedical dosimetry: relationship between low-level exposure and covalent binding of heterocyclic amine carcinogens to DNA. *Proc. Natl. Acad. Sci USA* 1990, *87*, 5288 – 5292.

[77] Dingley, K. H., Curtis, K. D., Nowell, S., Felton, J. S., *et al.*, DNA and protein adduct formation in the colon and blood of humans after exposure to a dietary-relevant dose of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. *Cancer Epidemiol. Biomarkers Prev.* 1999, *8*, 507 – 512.

[78] Mauthe, R. J., Dingley, K. H., Leveson, S. H., Freeman, S. P., *et al.*, Comparison of DNA-adduct and tissue-available dose levels of MeIQx in human and rodent colon following administration of a very low dose. *Int. J. Cancer* 1999, *80*, 539 – 545.

[79] Turteltaub, K. W., Mauthe, R. J., Dingley, K. H., Vogel, J. S., *et al.*, MeIQx-DNA adduct formation in rodent and human tissues at low doses. *Mutat. Res.* 1997, *376*, 243 – 252.

[80] Lightfoot, T. J., Coxhead, J. M., Cupid, B. C., Nicholson, S., Garner, R. C., Analysis of DNA adducts by accelerator mass spectrometry in human breast tissue after administration of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine and benzo[*a*]pyrene. *Mutat. Res.* 2000, *472*, 119 – 127.

[81] Schweikl, H., Taylor, J. A., Kitareewan, S., Linko, P., *et al.*, Expression of CYP1A1 and CYP1A2 genes in human liver. *Pharmacogenetics* 1993, *3*, 239 – 249.

[82] Hammons, G. J., Yan-Sanders, Y., Jin, B., Blann, E., *et al.*, Specific site methylation in the 5′-flanking region of CYP1A2 interindividual differences in human livers. *Life Sci.* 2001, *69*, 839 – 845.

[83] Belloc, C., Baird, S., Cosme, J., Lecoeur, S., *et al.*, Human cytochrome P450 expressed in *Escherichia coli*, production of specific antibodies. *Toxicology* 1996, *106*, 207 – 219.

[84] Conney, A. H., Induction of microsomal enzymes by foreign chemicals and carcinogenesis by polycyclic aromatic hydrocarbons: G. H. A. Clowes Memorial Lecture. *Cancer Res.* 1982, *42*, 4875 – 4917.

[85] Sesardic, D., Boobis, A. R., Edwards, R. J., Davies, D. S., A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the O-deethylation of phenacetin and is inducible by cigarette smoking. *Br. J. Clin. Pharmacol.* 1988, *26*, 363 – 372.

[86] Schrenk, D., Brockmeier, D., Morike, K., Bock, K. W., Eichelbaum, M., A distribution study of CYP1A2 phenotypes among smokers and non-smokers in a cohort of healthy Caucasian volunteers. *Eur. J. Clin. Pharmacol.* 1998, *53*, 361 – 367.

[87] Lampe, J. W., King, I. B., Li, S., Grate, M. T., *et al.*, Brassica vegetables increase and apiaceous vegetables decrease cytochrome P450 1A2 activity in humans: changes in caffeine metabolite ratios in response to controlled vegetable diets. *Carcinogenesis* 2000, *21*, 1157 – 1162.

[88] Murray, S., Lake, B. G., Gray, S., Edwards, A. J., *et al.*, Effect of cruciferous vegetable consumption on heterocyclic aromatic amine metabolism in man. *Carcinogenesis* 2001, *22*, 1413 – 1420.

[89] Sinha, R., Rothman, N., Brown, E. D., Mark, S. D., *et al.*, Pan-fried meat containing high levels of heterocyclic aromatic amines but low levels of polycyclic aromatic hydrocarbons induces cytochrome P4501A2 activity in humans. *Cancer Res.* 1994, *54*, 6154 – 6159.

[90] Nakajima, M., Yokoi, T., Mizutani, M., Kinoshita, M., *et al.*, Genetic polymorphism in the 5′-flanking region of human CYP1A2 gene: effect on the CYP1A2 inducibility in humans. *J. Biochem. (Tokyo)* 1999, *125*, 803 – 808.

[91] Sachse, C., Bhambra, U., Smith, G., Lightfoot, T. J., *et al.*, Polymorphisms in the cytochrome P450 CYP1A2 gene (CYP1A2) in colorectal cancer patients and controls: allele frequencies, linkage disequilibrium and influence on caffeine metabolism. *Br. J. Clin. Pharmacol.* 2003, *55*, 68 – 76.

[92] Butler, M. A., Guengerich, F. P., Kadlubar, F. F., Metabolic oxidation of the carcinogens 4-aminobiphenyl and 4,4′-methylenebis(2-chloroaniline) by human hepatic microsomes and by purified rat hepatic cytochrome P-450 monooxygenases. *Cancer Res.* 1989, *49*, 25 – 31.

[93] Chevalier, D., Cauffiez, C., Allorge, D., Lo-Guidice, J. M., *et al.*, Five novel natural allelic variants – 951A > C, 1042G > A (D348N), 1156A > T (I386F), 1217G > A (C406Y) and 1291C > T (C431Y) – of the human CYP1A2 gene in a French Caucasian population. *Hum. Mutat.* 2001, *17*, 355 – 356.

[94] Murayama, N., Soyama, A., Saito, Y., Nakajima, Y., *et al.*, Six novel nonsynonymous CYP1A2 gene polymorphisms: catalytic activities of the naturally occurring variant enzymes. *J. Pharmacol. Exp. Ther.* 2004, *308*, 300 – 306.

[95] Zhou, H., Josephy, P. D., Kim, D., Guengerich, F. P., Functional characterization of four allelic variants of human cytochrome P450 1A2. *Arch. Biochem. Biophys.* 2004, *422*, 23 – 30.

[96] Blanchard, R. L., Freimuth, R. R., Buck, J., Weinshilboum, R. M., Coughtrie, M. W., A proposed nomenclature system for the cytosolic sulfotransferase (SULT) superfamily. *Pharmacogenetics* 2004, *14*, 199 – 211.

[97] Nowell, S., Ambrosone, C. B., Ozawa, S., MacLeod, S. L., *et al.*, Relationship of phenol sulfotransferase activity (SULT1A1) genotype to sulfotransferase phenotype in platelet cytosol. *Pharmacogenetics* 2000, *10*, 789 – 797.

[98] Coles, B., Nowell, S. A., MacLeod, S. L., Sweeney, C., Lang, N. P., Kadlubar, F. F., The role of human glutathione *S*-transferases (hGSTs) in the detoxification of the food-derived carcinogen metabolite *N*-acetoxy-PhIP, and the effect of a polymorphism in hGSTA1 on colorectal cancer risk. *Mutat. Res.* 2001, *482*, 3 – 10.

[99] Hein, D. W., Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. *Mutat. Res.* 2002, *506 – 507*, 65 – 77.

[100] Wells, P. G., Mackenzie, P. I., Chowdhury, J. R., Guillemette, C., *et al.*, Glucuronidation and the UDP-glucuronosyltransferases in health and disease. *Drug Metab. Dispos.* 2004, *32*, 281 – 290.

[101] Hickman, D., Pope, J., Patil, S. D. F. G., Smelt, V., *et al.*, Expression of arylamine *N*-acetyltransferase in human intestine. *Gut* 1998, *42*, 402 – 409.

[102] Windmill, K. F., Gaedigk, A., Hall, P. M., Samaratunga, H., *et al.*, Localization of *N*-acetyltransferases NAT1 and NAT2 in human tissues. *Toxicol. Sci.* 2000, *54*, 19 – 29.

[103] Kadlubar, F. F., Beland, F. A., Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamides. In: Harvey, R. G. (Ed.), *Polycyclic Hydrocarbons and Carcinogenesis*, American Chemical Society, Washington, DC 1985, pp. 332 – 370.

[104] Shinohara, A., Yamazoe, Y., Saito, K., Kamataki, T., Kato, R., Species differences in the *N*-acetylation by liver cytosol of mutagenic heterocyclic aromatic amines in protein pyrolysates. *Carcinogenesis* 1984, *5*, 683 – 686.

[105] Ozawa, S., bu-Zeid, M., Kawakubo, Y., Toyama, S., *et al.*, Monomorphic and polymorphic isozymes of arylamine *N*-acetyltransferases in hamster liver: purification of the isozymes and genetic basis of *N*-acetylation polymorphism. *Carcinogenesis* 1990, *11*, 2137 – 2144.

[106] Minchin, R. F., Reeves, P. T., Teitel, C. H., McManus, M. E., *et al.*, *N*- and *O*-acetylation of aromatic and hetercyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in *COS-1* cells. *Biochem. Biophys. Res. Commun.* 1992, *185*, 839 – 844.

[107] Frandsen, H., Grivas, S., Andersson, R., Dragsted, L., Larsen, J. C., Reaction of the N^2-acetoxy derivative of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) with 2′-deoxyguanosine and DNA. Synthesis and identification of N^2-(2′-deoxyguanosin-8-yl)-PhIP. *Carcinogenesis* 1992, *13*, 629 – 635.

[108] King, R. S., Teitel, C. H., Kadlubar, F. F., *In vitro* bioactivation of *N*-hydroxy-2-amino-α-carboline. *Carcinogenesis* 2000, *21*, 1347 – 1354.

[109] Sadrieh, N., Davis, C. D., Snyderwine, E. G., *N*-Acetyltransferase expression and metabolic activation of the food-derived heterocyclic amines in the human mammary gland. *Cancer Res.* 1996, *56*, 2683 – 2687.

[110] Williams, J. A., Phillips, D. H., Mammary expression of xenobiotic metabolizing enzymes and their potential role in breast cancer. *Cancer Res.* 2000, *60*, 4667 – 4677.

[111] Williams, J. A., Stone, E. M., Fakis, G., Johnson, N., *et al.*, *N*-Acetyltransferases, sulfotransferases and heterocyclic amine activation in the breast. *Pharmacogenetics* 2001, *11*, 373 – 388.

[112] Wang, C. Y., Debiec-Rychter, M., Schut, H. A., Morse, P., *et al.*, *N*-Acetyltransferase expression and DNA binding of *N*-hydroxyheterocyclic amines in human prostate epithelium. *Carcinogenesis* 1999, *20*, 1591 – 1595.

[113] DeBruin, L. S., Martos, P. A., Josephy, P. D., Detection of PhIP (2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine) in the milk of healthy women. *Chem. Res. Toxicol.* 2001, *14*, 1523 – 1528.

[114] Zhu, J., Chang, P., Bondy, M. L., Sahin, A. A., *et al.*, Detection of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine-DNA adducts in normal breast tissues and risk of breast cancer. *Cancer Epidemiol. Biomarkers Prev.* 2003, *12*, 830 – 837.

[115] Lang, N. P., Butler, M. A., Massengill, J. P., Lawson, M., *et al.*, Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. *Cancer Epidemiol. Biomarkers Prev.* 1994, *3*, 675 – 682.

[116] Le Marchand, L., Hankin, J. H., Wilkens, L. R., Pierce, L. M., *et al.*, Combined effects of well-done red meat, smoking, and rapid *N*-acetyltransferase 2 and CYP1A2 phenotypes in increasing colorectal cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 1259 – 1266.

[117] Ishibe, N., Sinha, R., Hein, D. W., Kulldorff, M., *et al.*, Genetic polymorphisms in heterocyclic amine metabolism and risk of colorectal adenomas. *Pharmacogenetics* 2002, *12*, 145 – 150.

[118] Barrett, J. H., Smith, G., Waxman, R., Gooderham, N., *et al.*, Investigation of interaction between *N*-acetyltransferase 2 and heterocyclic amines as potential risk factors for colorectal cancer. *Carcinogenesis* 2003, *24*, 275 – 282.

[119] Tiemersma, E. W., Voskuil, D. W., Bunschoten, A., Hogendoorn, E. A., *et al.*, Risk of colorectal adenomas in relation to meat consumption, meat preparation, and genetic susceptibility in a Dutch population. *Cancer Causes Control* 2004, *15*, 225 – 236.

[120] Ozawa, S., Chou, H. C., Kadlubar, F. F., Nagata, K., *et al.*, Activation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine by cDNA-expressed human and rat arylsulfotransferases. *Jpn. J. Cancer Res.* 1994, *85*, 1220 – 1228.

[121] Muckel, E., Frandsen, H., Glatt, H. R., Heterologous expression of human *N*-acetyltransferases 1 and 2 and sulfotransferase 1A1 in *Salmonella typhimurium* for mutagenicity testing of heterocyclic amines. *Food Chem. Toxicol.* 2002, *40*, 1063 – 1068.

[122] Glatt, H., Pabel, U., Meinl, W., Frederiksen, H., *et al.*, Bioactivation of the heterocyclic aromatic amine 2-amino-3-methyl-9*H*-pyrido [2,3-*b*]indole (MeAalphaC) in recombinant test systems expressing human xenobiotic-metabolizing enzymes. *Carcinogenesis* 2004, *25*, 801 – 807.

[123] Chou, H.-C., Lang, N. P., Kadlubar, F. F., Metabolic activation of *N*-hydroxy arylamines and *N*-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res.* 1995, *55*, 525 – 529.

[124] Lewis, A. J., Walle, U. K., King, R. S., Kadlubar, F. F., *et al.*, Bioactivation of the cooked food mutagen *N*-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by estrogen sulfotransferase in cultured human mammary epithelial cells. *Carcinogenesis* 1998, *19*, 2049 – 2053.

[125] Raftogianis, R. B., Wood, T. C., Weinshilboum, R. M., Human phenol sulfotransferases SULT1A2 and SULT1A1: genetic polymorphisms, allozyme properties, and human liver genotype-phenotype correlations. *Biochem. Pharmacol.* 1999, *58*, 605 – 616.

[126] Glatt, H., Boeing, H., Engelke, C. E., Ma, L., *et al.*, Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. *Mutat. Res.* 2001, *482*, 27 – 40.

[127] Carlini, E. J., Raftogianis, R. B., Wood, T. C., Jin, F., *et al.*, Sulfation pharmacogenetics: SULT1A1 and SULT1A2 allele frequencies in Caucasian, Chinese and African-American subjects. *Pharmacogenetics* 2001, *11*, 57 – 68.

[128] Ozawa, S., Tang, Y. M., Yamazoe, Y., Kato, R., *et al.*, Genetic polymorphisms in human liver phenol sulfotransferases involved in the bioactivation of *N*-hydroxy derivatives of carcinogenic arylamines and heterocyclic amines. *Chem.-Biol. Interact.* 1998, *109*, 237 – 248.

[129] Zheng, W., Xie, D., Cerhan, J. R., Sellers, T. A., *et al.*, Sulfotransferase 1A1 polymorphism, endogenous estrogen exposure, well-done meat intake, and breast cancer risk. *Cancer Epidemiol. Biomarkers Prev.* 2001, *10*, 89 – 94.

[130] Burchell, B., Genetic variation of human UDP-glucuronosyltransferase: implications in disease and drug glucuronidation. *Am. J. Pharmacogenomics* 2003, *3*, 37 – 52.

[131] Nowell, S. A., Massengill, J. S., Williams, S., Radominska-Pandya, A., *et al.*, Glucuronidation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by human microsomal UDP-glucuronosyltransferases: identification of specific UGT1A family isoforms involved. *Carcinogenesis* 1999, *20*, 1107 – 1114.

[132] Strassburg, C. P., Strassburg, A., Nguyen, N., Li, Q., *et al.*, Regulation and function of family 1 and family 2 UDP-glucuronosyltransferase genes (UGT1A, UGT2B) in human oesophagus. *Biochem. J.* 1999, *338 (Pt 2)*, 489 – 498.

[133] Malfatti, M. A., Felton, J. S., *N*-Glucuronidation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and *N*-hydroxy-PhIP by specific human UDP-glucuronosyltransferases. *Carcinogenesis* 2001, *22*, 1087 – 1093.

[134] Stillwell, W. G., Turesky, R. J., Sinha, R., Tannenbaum, S. R., *N*-Oxidative metabolism of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in humans: excretion of the N^2-glucuronide conjugate of 2-hydroxyamino-MeIQx in urine. *Cancer Res.* 1999, *59*, 5154 – 5159.

[135] Hayes, J. D., Strange, R. C., Glutathione *S*-transferase polymorphisms and their biological consequences. *Pharmacology* 2000, *61*, 154 – 166.

[136] Eaton, D. L., Bammler, T. K., Concise review of the glutathione *S*-transferases and their significance to toxicology. *Toxicol. Sci.* 1999, *49*, 156 – 164.

[137] Hayes, J. D., Pulford, D. J., The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit. Rev. Biochem. Mol. Biol.* 1995, *30*, 445 – 600.

[138] Bogaards, J. J., Verhagen, H., Willems, M. I., van Poppel, G., van Bladeren, P. J., Consumption of Brussels sprouts results in elevated α-class glutathione *S*-transferase levels in human blood plasma. *Carcinogenesis* 1994, *15*, 1073 – 1075.

[139] Morel, F., Langouët, S., Maheo, K., Guillouzo, A., The use of primary hepatocyte cultures for the evaluation of chemoprotective agents. *Cell Biol. Toxicol.* 1997, *13*, 323 – 329.

[140] van Ommen, B., Bogaards, J. J., Peters, W. H., Blaauboer, B., van Bladeren, P. J., Quantification of human hepatic glutathione *S*-transferases. *Biochem. J.* 1990, *269*, 609 – 613.

[141] Meyer, D. J., Harris, J. M., Gilmore, K. S., Coles, B., *et al.*, Quantitation of tissue- and sex-specific induction of rat GSH transferase subunits by dietary 1,2-dithiole-3-thiones. *Carcinogenesis* 1993, *14*, 567 – 572.

[142] Meyer, D. J., Coles, B., Harris, J., Gilmore, K. S., *et al.*, Induction of rat liver GSH transferases by 1,2-dithiole-3-thione illustrates both anticarcinogenic and tumor-promoting properties. *Basic Life Sci.* 1993, *61*, 171 – 179.

[143] Coles, B. F., Morel, F., Rauch, C., Huber, W. W., *et al.*, Effect of polymorphism in the human glutathione *S*-transferase A1 promoter on hepatic GSTA1 and GSTA2 expression. *Pharmacogenetics* 2001, *11*, 663 – 669.

[144] Kaderlik, K. R., Minchin, R. F., Mulder, G. J., Ilett, K. F., *et al.*, Metabolic activation pathway for the formation of DNA adducts of the carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in rat extrahepatic tissues. *Carcinogenesis* 1994, *15*, 1703 – 1709.

[145] Strassburg, C. P., Manns, M. P., Tukey, R. H., Expression of the UDP-glucuronosyltransferase 1A locus in human colon. Identification and characterization of the novel extrahepatic UGT1A8. *J. Biol. Chem.* 1998, *273*, 8719 – 8726.

[146] Magagnotti, C., Pastorelli, R., Pozzi, S., Andreoni, B., *et al.*, Genetic polymorphisms and modulation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-DNA adducts in human lymphocytes. *Int. J. Cancer* 2003, *107*, 878 – 884.

[147] Gaylor, D. W., Kadlubar, F. F., Quantitative risk assessments of heterocyclic amines in cooked foods. In: Hayatsu, H. (Ed.), *Mutagens in Foods: Detection and Prevention*. CRC Press, Boca Raton, FL 1991, pp. 229 – 236.

[148] Bogen, K. T., Cancer potencies of heterocyclic amines found in cooked foods. *Food Chem. Toxicol.* 1994, *32*, 505 – 515.

[149] Layton, D. W., Bogen, K. T., Knize, M. G., Hatch, F. T., *et al.*, Cancer risk of heterocyclic amines in cooked foods: an analysis and implications for research. *Carcinogenesis* 1995, *16*, 39 – 52.

[150] Stavric, B., Biological significance of trace levels of mutagenic heterocyclic aromatic amines in human diet: A critical review. *Food Chem. Toxicol.* 1994, *32*, 977 – 994.

[151] Zimmerli, B., Rhyn, P., Zoller, O., Schlatter, J., Occurrence of heterocyclic aromatic amines in the Swiss diet: analytical method, exposure estimation and risk assessment. *Food Addit. Contam* 2001, *18*, 533 – 551.

[152] Adamson, R. H., Thorgeirsson, U. P., Sugimura, T., Extrapolation of heterocyclic amine carcinogenesis data from rodents and nonhuman primates to humans. *Arch. Toxicol. Suppl.* 1996, *18*, 303 – 318.

[153] Lutz, W. K., Schlatter, J., Chemical carcinogens and overnutrition in diet-related cancer. *Carcinogenesis* 1992, *13*, 2211 – 2216.

[154] Guy, P. A., Gremaud, E., Richoz, J., Turesky, R. J., Quantitative analysis of mutagenic heterocyclic aromatic amines in cooked meat using liquid chromatography-atmospheric pressure chemical ionisation tandem mass spectrometry. *J. Chromatogr. A* 2000, *883*, 89 – 102.

[155] Turesky, R. J., Box, R. M., Markovic, J., Gremaud, E., Snyderwine, E. G., Formation and persistence of DNA adducts of 2-amino-3-methylimidazo[4,5-*f*]quinoline in the rat and nonhuman primates. *Mutat. Res.* 1997, *376*, 235 – 241.

[156] Poirier, M. C., Beland, F. A., DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: Implications for DNA adduct-based human cancer risk assessment. *Chem. Res. Toxicol.* 1992, *5*, 749 – 755.

[157] Hemminki, K., DNA adducts, mutations and cancer. *Carcinogenesis* 1993, *14*, 2007 – 2012.

[158] Swenberg, J. A., La, D. K., Scheller, N. A., Wu, K. Y., Dose-response relationships for carcinogens. *Toxicol. Lett.* 1995, *82 – 83*, 751 – 756.

[159] Lutz, W. K., Dose-response relationships in chemical carcinogenesis: superposition of different mechanisms of action, resulting in linear-nonlinear curves, practical thresholds, J-shapes. *Mutat. Res.* 1998, *405*, 117 – 124.

[160] Sugimura, T., Mutagens, carcinogens, and tumor promoters in our daily food. *Cancer* 1982, *49*, 1970 – 1984.

[161] Ochiai, M., Watanabe, M., Kushida, H., Wakabayashi, K., *et al.*, DNA adduct formation, cell proliferation and aberrant crypt focus formation induced by PhIP in male and female rat colon with relevance to carcinogenesis. *Carcinogenesis* 1996, *17*, 95 – 98.

[162] Nagao, M., Ushijima, T., Toyota, M., Inoue, R., Sugimura, T., Genetic changes induced by heterocyclic amines. *Mutat. Res.* 1997, *376*, 161 – 167.

[163] Ayalogu, E. O., Snelling, J., Lewis, D. F., Talwar, S., *et al.*, Induction of hepatic CYP1A2 by the oral administration of caffeine to rats: lack of association with the Ah locus. *Biochim. Biophys. Acta* 1995, *1272*, 89 – 94.

[164] Chen, L., Bondoc, F. Y., Lee, M. J., Hussin, A. H., *et al.*, Caffeine induces cytochrome P4501A2: induction of CYP1A2 by tea in rats. *Drug Metab. Dispos.* 1996, *24*, 529 – 533.

[165] McArdle, N. J., Clifford, M. N., Ioannides, C., Consumption of tea modulates the urinary excretion of mutagens in rats treated with IQ. Role of caffeine. *Mutat. Res.* 1999, *441*, 191 – 203.

[166] Turesky, R. J., Richoz, J., Constable, A., Curtis, K. D., *et al.*, The effects of coffee on enzymes involved in metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in rats. *Chem.-Biol. Interact.* 2003, *145*, 251 – 265.

[167] Hagiwara, A., Boonyaphiphat, P., Tanaka, H., Kawabe, M., *et al.*, Organ-dependent modifying effects of caffeine, and two naturally occurring antioxidants α-tocopherol and n-tritriacontane-16,18-dione, on 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)- induced mammary and colonic carcinogenesis in female F344 rats. *Jpn. J. Cancer Res.* 1999, *90*, 399 – 405.

[168] Wattenberg, L. W., Inhibition of carcinogenesis by minor dietary constituents. *Cancer Res.* 1992, *52*, 2085s – 2091s.

[169] Talalay, P., Fahey, J. W., Phytochemicals from cruciferous plants protect against cancer by modulating carcinogen metabolism. *J. Nutr.* 2001, *131*, 3027S – 3033S.

[170] He, Y. H., Friesen, M. D., Ruch, R. J., Schut, H. A., Indole-3-carbinol as a chemopreventive agent in 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) carcinogenesis: inhibition of PhIP-DNA adduct formation, acceleration of PhIP metabolism, and induction of cytochrome P450 in female F344 rats. *Food Chem. Toxicol.* 2000, *38*, 15 – 23.

[171] Embola, C. W., Weisburger, J. H., Weisburger, M. C., Urinary excretion of N-OH-2-amino-3-methylimidazo[4,5-*f*]quinoline-*N*-glucuronide in F344 rats is enhanced by green tea. *Carcinogenesis* 2001, *22*, 1095 – 1098.

[172] Turesky, R. J., Richoz, J., Constable, A., Curtis, K. D., *et al.*, The effects of coffee on enzymes involved in metabolism of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in rats. *Chem.-Biol. Interact.* 2003, *145*, 251 – 265.

[173] Weisburger, J. H., Dolan, L., Pittman, B., Inhibition of PhIP mutagenicity by caffeine, lycopene, daidzein, and genistein. *Mutat. Res.* 1998, *416*, 125 – 128.

[174] Schut, H. A., Yao, R., Tea as a potential chemopreventive agent in PhIP carcinogenesis: effects of green tea and black tea on PhIP-DNA adduct formation in female F-344 rats. *Nutr. Cancer* 2000, *36*, 52 – 58.

[175] Liew, C., Schut, H. A., Chin, S. F., Pariza, M. W., Dashwood, R. H., Protection of conjugated linoleic acids against 2-amino-3- methylimidazo[4,5-*f*]quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. *Carcinogenesis* 1995, *16*, 3037 – 3043.

[176] Schut, H. A., Cummings, D. A., Smale, M. H., Josyula, S., Friesen, M. D., DNA adducts of heterocyclic amines: formation, removal and inhibition by dietary components. *Mutat. Res.* 1997, *376*, 185 – 194.

[177] Dashwood, R. H., Modulation of heterocyclic amine-induced mutagenicity and carcinogenicity: an 'A-to-Z' guide to chemopreventive agents, promoters, and transgenic models. *Mutat. Res.* 2002, *511*, 89 – 112.

[178] Schwab, C. E., Huber, W. W., Parzefall, W., Hietsch, G., *et al.*, Search for compounds that inhibit the genotoxic and carcinogenic effects of heterocyclic aromatic amines. *Crit. Rev. Toxicol.* 2000, *30*, 1 – 69.

[179] Dashwood, R., Guo, D., Protective properties of chlorophylls against the covalent binding of heterocyclic amines to DNA *in vitro* and *in vivo*. In: Adamson, R. H., Gustafsson, J.-A., Ito, N., Nagao, M., Sugimura, T., Wakabayashi, K., Yamazoe, Y. (Eds.), *Heterocyclic Amines in Cooked Foods: Possible Human Carcinogens. 23rd Proceedings of the Princess Takamatusu Cancer Society*, Princeton Scientific Publishing, Princeton, NJ 1995, pp. 181 – 189.

[180] Kulp, K. S., Fortson, S. L., Knize, M. G., Felton, J. S., An *in vitro* model system to predict the bioaccessibility of heterocyclic amines from a cooked meat matrix. *Food Chem. Toxicol.* 2003, *41*, 1701 – 1710.

[181] Sugimura, T., Nutrition and dietary carcinogens. *Carcinogenesis* 2000, *21*, 387 – 395.

[182] Nowell, S., Coles, B., Sinha, R., MacLeod, S., *et al.*, Analysis of total meat intake and exposure to individual heterocyclic amines in a case-control study of colorectal cancer: contribution of metabolic variation to risk. *Mutat. Res.* 2002, *506 – 507*, 175 – 185.

[183] Sweeney, C., Coles, B. F., Nowell, S., Lang, N. P., Kadlubar, F. F., Novel markers of susceptibility to carcinogens in diet: associations with colorectal cancer. *Toxicology* 2002, *181 – 182*, 83 – 87.

[184] Bingham, S. A., High-meat diets and cancer risk. *Proc. Nutr. Soc.* 1999, *58*, 243 – 248.

[185] Hughes, R., Cross, A. J., Pollock, J. R., Bingham, S., Dose-dependent effect of dietary meat on endogenous colonic *N*-nitrosation. *Carcinogenesis* 2001, *22*, 199 – 202.

[186] Lynch, A. M., Knize, M. G., Boobis, A. R., Gooderham, N., *et al.*, Intra- and interindividual variability in systemic exposure in humans to 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, carcinogens present in food. *Cancer Res.* 1992, *52*, 6216 – 6223.

[187] Stillwell, W. G., Turesky, R. J., Gross, G. A., Skipper, P. L., Tannenbaum, S. R., Human urinary excretion of sulfamate and glucuronde conjugates of 2-amino-3,8-dimethylimdazo[4,5-*f*]quinoxaline (MeIQx). *Cancer Epidemiol. Biomarkers Prev.* 1994, *3*, 399 – 405.

[188] Turesky, R. J., Vouros, P., Formation and analysis of heterocyclic aromatic amine-DNA adducts *in vitro* and *in vivo. J. Chromatogr. B* 2004, *802*, 155 – 166.

[189] Vineis, P., Martone, T., Genetic-environmental interactions and low-level exposure to carcinogens. *Epidemiology* 1995, *6*, 455 – 457.

[190] Vineis, P., The relationship between polymorphisms of xenobiotic metabolizing enzymes and susceptibility to cancer. *Toxicology* 2002, *181 – 182*, 457 – 462.

