LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

Susceptibility to Exposure to Heterocyclic Amines from Cooked Food: Role of UDP-glucuronosyltransferases

M. I A. Malfatti, J. S. Felton

August 24, 2005

Nutritional Genomics

Disclaimer

Susceptibility to Exposure to Heterocyclic Amines from Cooked Food: Role of

UDP-glucuronosyltransferases

Michael A. Malfatti and James S. Felton

Biosciences Program

Lawrence Livermore National Laboratory

Livermore, CA USA

Introduction

A number of carcinogenic heterocyclic amines (PhIP, MeIQx, and DiMeIQx) are produced from the condensation of creatinine, hexoses and amino acids during the cooking of meat (1). There are many variables that impact the production and subsequent ingestion of these compounds in our diet. Temperature, type of meat product, cooking method, doneness, and other factors affect the quantity of these carcinogens consumed by humans. Estimates of ingestion of these carcinogens are 1-20 ng/kg body weight per day (2). Human case control studies that correlate meat consumption from

well-done cooking practices with cancer incidence indicate excess tumors for breast, colon, stomach, esophagus, and possibly prostate (3-5).

Genetic Susceptibility

Heterocyclic amines (HAs) are activated and detoxified through a number of different pathways. The initial *N*-oxidation at the exocyclic amino group, present in all HAs, primarily occurs in the liver, and almost exclusively by cytochrome P4501A2 (6, 7). Further activation is possibly by conjugating enzymes like *N*-acetyltransferase and sulfotransferase to eventually give a very reactive nitrenium ion that theoretically binds to the guanine of DNA at the C-8 position (8). This resulting adduct then leads to mutations and cancer if not removed by the nucleotide excision repair pathway. If precursors to the reactive intermediates can be removed from the cells by detoxification, then the amount of DNA damage can be minimized. UDP-glucuronysltransferase appears to fulfill this role. Although genetic variants in any of the activation pathways can have an impact on the total amount of DNA damage, we believe a major influence on the whole pathway and thus susceptibility to cancer is the ability to detoxify. We have described here the basics of this pathway (genes to protein products) and discussed the impact of variation of the numerous UGTs on DNA damage and ultimately cancer.

UDP-glucuronosyltransferase

The elimination of many endogenous and xenobiotic chemicals from the body is highly dependent upon UDP-glucuronosyltransferase (UGT)-dependent conjugation with glucuronic acid. Compared to other conjugating enzymatic reactions UGT conjugation is

somewhat unique. The most notable differences are the location of the UGT protein and the atypical gene structure of one of the enzyme subfamilies. Similar to the cytochrome P450s, the UGTs have a wide and varied substrate selectivity and specificity. This trait makes them able to conjugate many types of chemical classes. UGTs are widely distributed among tissues making site-specific metabolism an important factor in UGT-mediated conjugation. Chemical inducers and repressors, as well as, genetic variation in some UGT genes regulate UGT expression. Furthermore, alterations in gene expression, due to genetic polymorphisms, can have profound effects on glucuronidation capacity. The metabolism of several chemical carcinogens, including the cooked-food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), can be influenced by the differential expression of certain UGT isozymes. This differential expression of UGT isoforms can change the metabolic ratio between bioactivation and detoxification. A change favoring bioactivation, caused by decreased glucuronidation activity, would likely lead to an increase in the susceptibility of potential tumor formation from carcinogen exposure.

The metabolism of certain chemical carcinogens by UGT-mediated glucuronidation is one of the central pathways in maintaining health. . The structure, function and especially regulation of specific UGTs all contribute to how chemical carcinogens can be bioactivated or detoxified. Differential glucuronidation capacity affects the bioactivation of the cooked-food carcinogen PhIP.

UDP-glucuronosyltransferase Biochemistry

The *UGT*s are a multigene superfamily of constitutively and inducible membrane bound enzymes that participate in the biotransformation of many different chemical compounds. Glucuronidation is an especially important pathway for detoxifying reactive intermediates from metabolic reactions, which otherwise can be biotransformed into cytotoxic or carcinogenic species (9). The UGTs catalyze the conjugation of glucuronic acid to a nucleophilic substrate that increases the polarity of the substrate to facilitate its excretion through the urine or bile (10, 11). The sugar co-substrate for the reaction is uridine 5'-diphosphoglucuronic acid (UDPGA), which is generated in the cytosol in a series of reactions starting with glucose. The rate-limiting enzyme in the UDPGA synthetic pathway is UDP-glucose dehydrogenase, and is most likely an important factor affecting the rate of glucuronidation (12). The subcellular localization of the UGTs is within the endoplasmic reticulum in a conformation such that the majority of the protein is luminal. This luminal localization results in the phenomenon of latency, possibly due to the ER membrane acting as a diffusional barrier for substrate and cofactor access, and metabolite and byproduct removal (13).

UDP-glucuronosyltransferase gene structure

The human UGT proteins are divided into two gene families, *UGT1* and *UGT2*, based on sequence homologies. These families are further divided into three subfamilies, *UGT1A, UGT2A, and UGT2B*. The proteins range from 529 to 534 amino acids in length for a molecular weight of 52-57 kDa, with several highly conserved regions that are important for membrane targeting and activity. The carboxyl terminus of all UGTs

shares a high degree of similarity whereas the amino-terminal domain is divergent. The *UGT1A* family is located on chromosome 2 (2q37) and is derived from a single gene locus composed of 5 exons (Figure 1). The amino half of the gene (280 amino acids) is encoded by one of thirteen exon 1 sequences that produce individual UGT1A proteins (14). Each unique exon 1 sequence is proximal to its own distinctive promoter. Of the thirteen exon 1 sequences, nine code for functional UGT proteins (UGT1A1, UGT1A3, UGT1A4, UGT1A5, UGT1A6, UGT1A7, UGT1A8, UGT1A9, UGT1A10) and four represent pseudogenes (UGT1A2p, UGT1A11p, UGT1A12p, UGT1A13p) (14, 15). The regulatory sequences flanking each of the exon 1 regions are thought to dictate the individual expression profile of each UGT1A isoform (16). The carboxyl terminus sequence (245 amino acids) is identical for all UGT1A subfamily members and is comprised of exons 2-5. This gene structure is atypical among enzymes involved in xenobiotic metabolism (12). It has been proposed that the unique amino-terminal half of each UGT protein codes for the specific substrate-binding domain while the common carboxyl-terminal half codes for the UDPGA co-substrate binding domain (17, 18). The existence of the unique substrate binding domains provides for the large substrate specificity and selectivity observed in the UGT1A proteins. In contrast to the UGT1 family, the UGT2 genes are located on chromosome 4 (4q13) and are comprised of eight individual structural genes (6 genes for *UGT2B*; 2 genes for *UGT2A* subfamilies). Like the UGT1A proteins, the UGT2B proteins share a high degree of similarity at the carboxyl-terminal region and are divergent at the amino-terminal domain. The UGT2B proteins are primarily responsible for steroid metabolism (19).

Substrate specificity and selectivity

The UGTs have wide and overlapping substrate specificities and selectivities. The substrates include endogenous steroids, hormones, bilirubin, bile acids, dietary constituents, and numerous xenobiotic drugs, environmental pollutants, and carcinogens. Functional groups known to be conjugated include phenols and aliphatic alcohols, carboxylic acids, primary, secondary and tertiary amines, and nucleophilic carbon atoms. A listing of UGT glucuronidation activity toward specific substrate classes for each UGT isozyme can be found in a review by Tukey and Strassburg (19). Glucuronidation is generally thought of as a detoxification reaction, however, there are examples were glucuronide conjugation results in increased biological activity. These include the *N-O*-glucuronides of hydroxamic acids and the acyl glucuronides of carboxylic acids. In addition, several drugs are known to produce glucuronide conjugates that have pharmacological or toxic activities higher than their parent compound. Examples of these include glucuronides of morphine, all-*trans*-retinol and all-*trans*-retinoic acid, and natural and synthetic estrogens (*reviewed in* (12)).

Tissue distribution of UDP-glucuronosyltransferase

Expression of UGT proteins has been detected in multiple tissues, with the liver being regarded as the site with the greatest glucuronidation capacity. High levels of activity have also been observed in the kidney, and intestine indicating a significant capacity for extrahepatic glucuronidation. Other tissues expressing UGT activity include lung, olfactory epithelium, ovary, mammary gland, testis, and prostate (*reviewed in* (19)). The mechanisms that determine the wide UGT tissue distribution and expression have yet

to be determined. In addition to having a wide tissue distribution, UGT proteins can be preferentially expressed in different tissues. The *UGT1A* gene locus in the liver codes for *UGT1A1, UGT1A3, UGT1A4, UGT1A6*, and *UGT1A9*. Expression of *UGT1A7*, *UGT1A8*, and *UGT1A10* is found exclusively in extrahepatic tissue, primarily in gastric, colon, and biliary tissue, respectively. Expression of the *UGT1A* locus in the colon is the most diverse with gene transcripts detected for *UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A8, UGT1A9,* and *UGT1A10*. The differential expression of the *UGT1A* family of enzymes demonstrates the importance of site-specific metabolism and substrate selectivity of the UGT1A proteins. Low expression of a particular UGT in a specific tissue could alter the metabolism of a xenobiotic compound in that tissue, potentially diverting it to pathways that would produce a more biologically active compound that could bind proteins and/or DNA.

Gene regulation

   Expression of *UGT* genes can be regulated by chemically mediated induction or repression. Studies have shown that human *UGT1A1, UGT1A6*, and *UGT1A9* expression is induced by dioxin via binding of the aryl hydrocarbon receptor (AhR) to a xenobiotic response element (20, 21). Upon ligand binding the AhR/ligand complex translocates to the nucleus where it dimerizes with the AhR nuclear translocator (ARNT). This heterodimer complex binds to a dioxin response element which results in enhanced *UGT* transcription and subsequent expression. Other inducers include antioxidants such as tertbutylhydroquinone and quercetin that can induce *UGT1A6* (22). *UGT1A1* has been shown to be up-regulated by 3-methylcholanthrene and oltripaz in human hepatocytes

and by flavonoids in human HepG2 cells (23, 24). Furthermore, the dietary anticarcinogens coumarin, curcumin, α-angelicalactone, fumaric acid and flavones caused an increase in the glucuronidation of 4-nitrophenol and 4-methylumbelliferone in rat hepatic microsomes (25).

More recently the human orphan nuclear receptors, human pregnane X receptor (hPXR) and constitutive androstane receptor (CAR), have been implicated in the regulation of *UGT* genes. Studies have shown that phenobarbital and rifampicin, which are ligands for hPXR and CAR, can mediate *UGT* expression. In human HepG2 cells it was shown that CAR binds to a nuclear response element within the *UGT1A1* promoter and enhances *UGT1A1* gene transcription. This enhancement is increased by phenobarbital and decreased by androstenol (an inhibitory ligand for CAR) (26). Deletion analysis of the *UGT1A1* promoter region resulted in the identification of a regulatory sequence that conferred hPXR regulation of the *UGT1A1* gene (27). Western blot analysis showed that both hPXR and CAR induce UGT1A1 and UGT1A6 activities. In addition, transgenic mice that possess a constitutively activated form of hPXR, demonstrated a significant increase in UGT activity towards steroids, carcinogens, and enhanced bilirubin clearance. Evidence is also suggests *UGT1A1* and *UGT1A6* are direct transcriptional targets for hPXR (27).

In addition to chemical induction of *UGT*s, repression of UGT activity has been reported for a number of chemical compounds. The antibiotic novobiocin was shown to inhibit UGT-mediated bilirubin conjugation in rats by disrupting Mg^{2+} complexes. This inhibition was dose dependent and caused hyperbilirubinemia in the animals, and was reversible when novobiocin was removed (28). In other studies, long chain acyl-CoAs,

which are intermediates in fatty acid metabolism pathways, have been shown to be excellent inhibitors of glucuronidation. Both oleoyl-CoA and palmityl-CoA caused a dose dependent inhibition of UGT activity in rat liver microsomes and hepatocytes. This inhibition was noncompetitive and occurred at physiological concentrations of acyl-CoAs (29, 30). This inhibition by fatty acid metabolites illustrates how complex the whole organism detoxification can be for dietary constituents. If this has physiological significance, well-done meats with higher fat content will not only have higher HA content but the detoxification pathways will be inhibited leading to increased DNA binding and cancer. Furthermore, significant down-regulation of hepatic *UGT1A1, UGT1A3, UGT1A4*, and *UGT1A9* has been reported in malignant hepatocellular carcinoma and its premalignant precursor, hepatic adenoma, but not in benign focal nodular hyperplasia (31). This finding indicates that down-regulation of the *UGT1A* gene is an early event in hepatocarcinogenesis.

Genetic variation

UGT activity can also be regulated by genetic variability. Interindividual expression patterns have been reported for all *UGT*s except *UGT1A10*. This variation in expression was observed only in extrahepatic tissue, whereas hepatic expression showed no difference in the expression of *UGT* gene transcripts. (32-35). Polymorphisms in *UGT1A1, UGT1A6, UGT1A7, UGT2B4, UGT2B7* and *UGT2B15* have been reported. These polymorphisms have been implicated as risk factors for certain clinical diseases and cancers (19, 35-40). The most notable polymorphisms are variants in the *UGT1A1* gene that result in significant down regulation of *UGT1A1* activity. UGT1A1 is involved

in the glucuronidation of: estradiol, simple and complex phenols, several chemical carcinogens, and is the only UGT isoform known to catalyze the glucuronidation of bilirubin in humans (19, 41, 42). Bilirubin is a toxic breakdown product of heme that can accumulate in tissues resulting in jaundice if not eliminated through transport via albumin binding or conjugation by UGT1A1. At high serum levels, bilirubin can cross the blood brain barrier and lead to fatal necrosis of neurons and glial tissue. Therefore, down regulation of *UGT1A1* activity can result in an increase in serum levels of unconjugated bilirubin which can lead to bilirubin toxicity.

In humans three forms of inheritable unconjugated hyperbilirubinemia exist. The most serious, although very rare, is Crigler-Najjar syndrome type I which is transmitted as an autosomal recessive trait in humans and is characterized by an inability to form bilirubin glucuronides. This condition is caused by mutant coding regions in various *UGT1A1* alleles which results in either a lack of UGT1A1 production or the production of a non-functional protein (*reviewed in* (19)). The onset of Crigler-Najjar type I results in early childhood death. Crigler-Najjar syndrome type II is less severe than type I and is characterized by having very low UGT1A1 activity (10% of normal activity). This condition can be treated with enzyme induction therapy using phenobarbital. The most common form of hyperbilirubinemia is Gilbert's syndrome, which occurs, in 3-10% of the general population. This condition is characterized by chronic, mild hyperbilirubinemia which is exacerbated by stress, infection, fasting or physical activity (19). Gilbert's syndrome is characterized by an allelic variant in the *UGT1A1* gene which contains an additional (TA) dinucleotide repeat in the "A(TA)$_n$TAA" box region of the promoter. (43, 44). Functional studies have shown that the reference *UGT1A*1

activity is associated with six TA repeats (*UGT1A1*1*). Increasing the number of TA

repeats leads to a decrease in the rate of *UGT1A1* transcription (35). The most common

variant allele contains seven TA repeats (*UGT1A1*28*) and is the polymorphism

associated with Gilbert's syndrome (16). This polymorphism results in hepatic bilirubin

UGT conjugation being reduced to about 30% of normal (13, 45). There is evidence to

suggest that individuals with Gilbert's syndrome may be at greater risk from toxicants

and carcinogens that are conjugated by UGT1A1 because their ability to detoxify these

compounds would be diminished (35). For example, a recent study has reported a

correlation between the *UGT1A1* "ATATAA" box polymorphism and a decreased ability

to glucuronidate and detoxify benzo[*a*]pyrene-*trans*7R,8R-dihydrodiol [BAPD($-$)] in

human liver microsomes (46). Although we have been primarily discussing HAs, PAHs,

such as BaP, are accumulated on the surface of meat when the meat is cooked on a

barbeque grill. As the fat drips onto the coals, it is then pyrolized to PAHs. The PAHs

are then deposited on the meat from the smoke plume. Clearly, dual exposure to both

classes of carcinogens is probably additive with respect to DNA damage, but specific

genetic variants as described above can attenuate the exposure. Subjects possessing the

UGT1A1*28 allelic variant (which contains $(TA)_7$ repeats) showed significant decreases

in UGT1A1 protein expression and BAPD($-$) glucuronidation activity in liver

microsomes when compared to subjects having the wild-type UGT1A1(*1/*1) genotype.

Other significant polymorphisms include allelic variation in *UGT1A6, UGT1A7,*

UGT2B7, and *UGT2B15*. *UGT1A6* has been found to be polymorphic with at least four

alleles characterized by three single nucleotide polymorphisms (SNPs) in the coding

region (19, 47). UGT1A6 catalyzes the conjugation of simple phenols and planar

arylamines, as well as many drugs including antidepressants and β-adrenoceptor blockers. The *UGT1A7* gene that is not expressed in liver is responsible for glucuronidating many different drugs and toxicants, including several carcinogens, contains 9 different variant alleles (16). One of the alleles (*UGT1A7*3*) is found in 17% of the general population. All the mutations in both the *UGT1A6* and *UGT1A7* genes are associated with lower glucuronidation activity compared to the wild type genotype (16). Both UGT2B7 and UGT2B15 catalyze the glucuronidation of steroid hormones, as well as several classes of xenobiotic substrates (11). Allelic variants have been identified for UGT2B7 and UGT2B15 isozymes resulting in a histidine to tyrosine and an aspartic acid to tyrosine amino-acid substitution, respectively (16, 48). *In vitro* studies have reported no difference in catalytic activity between the UGT2B7 and UGT2B15 reference alleles and the variant alleles (49, 50). *In vivo* studies have yet to be completed.

UDP-glucuronosyltransferase and cancer susceptibility

Polymorphisms in *UGT* genes have also been associated with a potential increase in the susceptibility to certain forms of cancer due to a reduced capacity to detoxify carcinogenic compounds. Several chemical carcinogens have been shown to be substrates for glucuronidation, including many primary amines, several BaPs, and some heterocyclic amines (*reviewed in* (19)). Studies have shown that a reduced glucuronidation capacity can lead to an increase in bioactivation for these compounds. For example, when *UGT1A* deficient Gunn rats were exposed to BaP, covalent binding to hepatic DNA and microsomal protein was enhanced, and production of BaP glucuronide conjugates were reduced when compared to rats with normal UGT1A activity (51). In

human tissue, glucuronidating activity towards benzo[*a*]pyrene-*trans*7*R*,8*R*-dihydrodiol [BAPD(—)] was compared with liver microsomes from humans expressing *UGT1A1*28* (Gilbert's Syndrome) versus normal liver microsomes. Results showed a significant decreased in BAPD(—) glucuronide conjugate formation in the subjects possessing the *UGT1A1*28* genotype (46). In another study, an over 200-fold interindividual variability was observed in both the glucuronidation and covalent binding of BaP metabolites in human lymphocytes exposed to BaP (52). A decrease in BaP glucuronidation activity correlated with both an increase in BaP covalent binding and enhanced BaP cytotoxicity. Reduced UGT activity can also alter the biotransformation of carcinogenic heterocyclic amines that are found in the diet.

Heterocyclic amine carcinogens in food

Diet has long been associated with cancer etiology (53-55). This association may be related to such nutritional factors as fat or fiber intake, antioxidant exposure, or exposure to carcinogenic substances present in the diet. Historically sources of mutagens/carcinogens in food have been derived from pesticides and artificially added chemicals such as food preservatives and coloring agents. In addition to synthetic chemicals, naturally occurring mutagens can be present in certain foods as well. These include pyrrolizidine alkaloids which occur in many plant species, hydrazines found in mushrooms, alkylating agents found in spice oils, and nitrites which produce nitrosamines from the degradation products of proteins or other food components (56). A more recently discovered source of food-derived mutagens/carcinogens are those produced during the cooking of food (57). Carcinogenic compounds derived from

13

cooking food include pyrolysis products which are formed at temperatures of 300°C to 600°C and low temperature (<300°C) thermic mutagens which are produced in high protein foods derived from muscle. One of the first observations of the carcinogenic potential of cooked-foods was made in 1939 by Widmark who reported malignant tumors in the mammary glands of female mice chronically exposed to extracts of horse muscle cooked at a temperature of 275°C (58). It was not until the mid to late 1970s, however, when better analytical techniques were developed that significant advances were made in the study of dietary thermic mutagens. These studies ultimately lead to the discovery of a class of heterocyclic amines (HAs) that are formed during the cooking of foods that are commonly consumed in a typical Western diet (57, 59, 60). These compounds are part of the amino-imidazoazaarene (AIA) class of HAs due to a common imidazole-ring structure and exocyclic amine group (61) (Figure 2). They are formed by the condensation of creatinine with amino acids during the cooking of meat, under normal household cooking conditions. The concentration of HAs found in cooked meats can range from less than 1 part per billion (ppb) to greater than 500 ppb depending on meat type, precursor concentration and cooking method (1, 2). In general, frying or grilling at high temperatures for longer time periods will increase the amount of HAs in meats.

These AIA HAs are among the most potent mutagens ever tested in the Ames/*Salmonella* mutation assay (57). In rodents, these compounds produced tumors in a variety of tissues including the liver, lung, intestine, breast, and prostate (62) In humans they have been shown to cause DNA adducts in multiple tissues (63-65). Epidemiology studies have indicated an increase risk of colon tumors associated with HA exposure from well-done red meat consumption (3, 66).

Carcinogenicity of PhIP

Of all the HAs currently identified, PhIP is the most mass abundant and has been detected at the highest levels in grilled or fried beef and chicken (2, 67). PhIP has been shown to be carcinogenic in both mice and rats. Exposure to PhIP produced lymphomas in the mesenteric lymph nodes, mediastinal lymph nodes and spleen of CDF1 mice exposed to 400 parts per million dietary PhIP for 579 days (68), as well as hepatic adenomas in neonatal mice (69). In F344 rats PhIP produced high incidences of colon, mammary and prostate carcinomas when administered at a concentration of 400 parts per million in the diet for 365 days (4, 5). PhIP has also been shown to induce DNA strand breaks and sister-chromatid exchanges in Chinese hamster ovary cells (70, 71), and to form DNA adducts in both rodent and human tissues (63, 72-75). In addition, intake of PhIP from well-done red meat consumption has been associated with an increased risk for breast cancer in women (76). These findings, together with the relative abundance of PhIP in cooked foods, indicate that PhIP may pose a significant risk to the development of certain human cancers.

Metabolism of PhIP

The metabolism of PhIP involves both phase I and phase II pathways for bioactivation and/or detoxification (Figure 3). PhIP bioactivation is highly dependent upon the hepatic cytochrome P4501A2 (*CYP1A2*) mediated *N*-hydroxylation to the corresponding 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (*N*-hydroxy-PhIP) (6, 7). In extrahepatic tissue CYP1A1 and CYP1B1 have also been reported to

activate PhIP (77). *N*-Hydroxy-PhIP is subsequently esterified by phase II sulfotransferases and/or acetyltransferases that generate the highly electrophilic *O*-sulfonyl and *O*-acetyl esters, respectively. These esters are capable of heterolytic cleavage to generate the reactive nitrinium ion which is considered the ultimate carcinogenic species(78). This reactive nitrinium ion is able to form DNA adducts, mainly at the C8 position of guanine (8, 72, 79). Other conjugating enzymes have also been shown to further activate *N*-hydroxy-PhIP and bind DNA but are considered minor contributors to PhIP bioactivation (78, 80). *N*-Glucuronidation can compete with these activation reactions resulting in the formation of the less reactive *N*-hydroxy-PhIP-N^2-glucuornide and *N*-hydroxy-PhIP-*N*3-glucuonide. These compounds can be excreted through the urine or bile, or can be transported to extrahepatic tissue where deconjugation by β-glucuronidase, can occur leading to the re-generation of the reactive intermediate *N*-hydroxy-PhIP (81, 82). Detoxification of PhIP involves formation of the CYP450-mediated non-reactive 2-amino-1-methyl-6-(4'-hydroxy)phenylimidazo[4,5-*b*]pyridine (4'-hydroxy-PhIP). This compound can undergo sulfotransferase- and/or glucuronosyltransferase-mediated conjugation, producing more polar unreactive compounds which are readily excreted (83, 84). This pathway is more prevalent in rodents than in humans because the rate of 4'-hydroxy-PhIP formation is much more dependent on CYP1A1 than CYP1A2, and CYP1A1 is not expressed in human liver, the site most responsible for PhIP hydroxylation (77, 85, 86). PhIP can also form nonreactive direct glucuronides at the N^2 and *N*3 positions (87).

In humans, studies have indicated that CYP1A2 catalyzed *N*-hydroxylation and subsequent UGT-mediated glucuronidation is quantitatively the most important pathway

in the metabolism of PhIP. When human volunteers were exposed to PhIP, N-hydroxy-

PhIP glucuronide conjugates accounted for approximately 60% of the total PhIP urinary

metabolites (88). N-hydroxy-PhIP-N^2-glucuronide was also the major metabolite in

human urine after consumption of a single cooked chicken meal (89). Further

investigations have determined that the $UGT1A$ subfamily of UGTs plays a major role in

the glucuronidation of PhIP (90-92). Microsomes containing human UGT1A isoforms

were able to convert N-hydroxy-PhIP to both N-hydroxy-PhIP-N^2-glucuronide and N-

hydroxy-PhIP-$N3$-glucuronide. A more recent study has implicated UGT1A1 as the

primary UGT1A isoform responsible for PhIP glucuronidation (93). Microsomal

preparations from $UGT1A1$ expressing baculovirus infected insect cells produced 5 times

more N-hydroxy-PhIP-N^2-glucuornide compared to microsomes containing UGT1A4, the

next most active UGT protein.

UDP-glucuronosyltransferase and PhIP risk susceptibility

It is difficult to assess PhIP risk susceptibility in humans due to wide variations in

PhIP metabolism. There are numerous factors that can influence metabolism which are

regarded as important determinants of individual susceptibility to the carcinogenic effects

of PhIP. These include but are not limited to PhIP dose, variations in diet, species

differences in metabolism, and polymorphic distribution of PhIP-metabolizing enzymes.

High doses of PhIP, typically used in animal studies, can potentially saturate enzymatic

pathways which can divert PhIP to metabolic pathways that would not usually be used in

a low dose exposure. Species differences in metabolism can also effect risk susceptibility

determinations. In humans and dogs UGT-mediated N-hydroxy-PhIP glucuronidation is

a major pathway in PhIP biotransformation, whereas in rodents this is a minor pathway (Figure 1.4). Polymorphic expression of PhIP-metabolizing enzymes can play a large role in determining risk susceptibility. These polymorphisms can arise from both heritable and environmental factors (94). Humans display a large interindividual variation in the expression of CYP450 and several of the phase II conjugation enzymes (*reviewed in* (95)). Studies have shown that the polymorphic expression of certain UGTs can lead to differential metabolism of both endogenous and exogenous substrates including PhIP (13). For example, when *UGT1A* deficient Gunn rats were dosed orally with PhIP, a decrease in PhIP glucuronide levels in the urine of these rats correlated with an increase in hepatic DNA adducts compared to control rats with normal UGT1A activity (96). PhIP glucuronides in the bile of *UGT1A* deficient rats dosed with PhIP intravenously were also reduced compared to control animals (97). Furthermore, when Chinese hamster ovary cells were transfected with the human *UGT1A1* gene a significant reduction in PhIP-induced cytotoxicity and mutation induction was observed when compared to control cells that did not contain the *UGT1A1* gene (98). These results suggest that UGT1A1 plays a major role in the metabolism of PhIP by providing a protective effect against PhIP induced toxicity and mutation induction, and that variations in UGT protein expression can potentially alter the bioactivation of PhIP.

Summary

Since UGT-mediated *N*-hydroxy-PhIP-glucuronidation is such a prominent metabolic step in the biotransformation of PhIP in humans, understanding the mechanistic aspects of PhIP glucuronidation and identifying the specific UGT enzymes

involved, and their regulation, is especially important. The failure to conjugate *N*-hydroxy-PhIP by glucuronidation could lead to further activation by sulfotransferase and/or acetyltransferase, resulting in highly reactive esters that can bind DNA, and potentially cause mutations. Furthermore, differential expression of UGT isoforms in specific tissues can change the metabolic ratio between bioactivation and detoxification. A change favoring bioactivation, due to decreased glucuronidation activity, would likely lead to an increase in the susceptibility of potential tumor formation from PhIP exposure. Knowing the glucuronidation capacity of the specific UGT isozymes involved in *N*-hydroxy-PhIP glucuronidation, and understanding the role of UGT in PhIP metabolism will allow for a better understanding of the overall bioactivation/detoxification mechanisms of PhIP. This will help in evaluating the individual susceptibility to the potential cancer risks associated with exposure to PhIP. It is hypothesized that individuals with low levels of specific UGT proteins will have a diminished capacity to detoxify PhIP, making them more susceptible to the deleterious effects from PhIP exposure.

Acknowledgements

References

1. Felton, J. S. and Knize, M. G. Heterocyclic-amine mutagens/carcinogens in foods. *In:* C. S. Cooper and P. L. Grover (eds.), Handbook of Experimental Pharmacology, pp. 471-502. Berlin-Heidelberg: Springer-Verlag, 1990.

2. Sinha, R., Rothman, N., Brown, E. D., Salmon, C. P., Knize, M. G., Swanson, C. A., Rossi, S. C., Mark, S. D., Levander, O. A., and Felton, J. S. 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 Research, *55:* 4516-4519, 1995.

3. Sinha, R. and Rothman, N. Role of well-done, grilled red meat, heterocyclic amines (HCAs) in the etiology of human cancer. Cancer Letters, *143:* 189-194, 1999.

4. Ito, N., Hasegawa, R., Sano, M., Tamano, S., Esumi, H., Takayama, S., and Sugimura, T. A new colon and mammary carcinogen in cooked food, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) . Carcinogenesis, *12:* 1503-1506, 1991.

5. Shirai, T., Sano, M., Tamano, S., Takahashi, S., Hirose, M., Futakuchi, M., Hasegawa, R., Imaida, K., Matsumoto, K., Wakabayashi, K., Sugimura, T., and Ito, N. The Prostate: A target for carcinogenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) derived from cooked foods. Cancer Research, *57:* 195-198, 1997.

6. Edwards, R. J., Murray, B. P., Murray, S., Schulz, T., Neubert, D., Gant, T. W., Thorgeirsson, S. S., Boobis, A. R., and Davies, D. S. Contribution of CYP1A1 and CYP1A2 to the activation of heterocyclic amines in monkeys and humans. Carcinogenesis, *15:* 829-836, 1994.

7. Boobis, A. R., Lynch, A. M., Murray, S., de la Torre, R., Solans, A., Farre, M., Segura, J., Gooderham, N. J., and Davies, D. S. CYP1A2-catalyzed conversion of dietary heterocyclic amines to their proximate carcinogens is their major route of metabolism in humans. Cancer Research, *54:* 89-94, 1994.

8. Nagaoka, H., Wakabayashi, K., Kim, S. B., Kim, I. S., Tanaka, Y., Ochiai, M., Tada, A., Nukaya, H., Sugimura, T., and Nagao, M. Adduct formation at C-8 of guanine on in vitro reaction of the ultamate form of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine with 2'-deoxyguanosine and its phosphate esters. Japan Journal of Cancer Research, *83:* 1025-1029, 1992.

9. Dutton, G. J. Glucuronidation of drugs and other compounds. Boca Raton: CRC Press, 1980.

10. Meech, R. and Mackenzie, P. I. Structure and function of uridinediphosphate glucurononosyltransferases. Clin. Exp. Pharmacol. Physiol., *24:* 907-915, 1997.

11. Radominska-Pandya, A., Czernik, P. J., Little, J. M., Battaglia, E., and Mackenzie, P. I. Structural and functional studies of UDP-glucuronosyltransferases. Drug Metabolism Reviews, *31:* 817-899, 1999.

12. Ritter, J. K. Roles of glucuronidation and UDP-glucuronosyltransferases in xenobiotic bioactivation reactions. Chemico-Biological Interactions, *129:* 171-193, 2000.

13. Fisher, M. B., Paine, M. F., Strelevitz, T. J., and Wrighton, S. A. The role of hepatic and extrahepatic UDP-glucuronosyltransferase in human drug metabolism. Drug Metabolism Reviews, *33:* 273-297, 2001.

14. Gong, Q. H., CHO, J. W., Huang, T., Potter, C., Gholami, N., Basu, N. K., Kubota, S., Carvalho, S., Pennington, M. W., Owens, I. S., and Popescu, N. C. Thirteen UDP-glucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. Pharmacogenetics, *11:* 357-368, 2001.

15. Owens, I. S. and Ritter, J. K. Gene structure at the human UGT1 locus creates diversity in isozyme structure, substrate specificity, and regulation. Prog. Nucleic Acid Res. Mol. Biol., *51:* 305-338, 1995.

16. Guillemette, C. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. The Pharmacogenomics Journal, *3:* 136-158, 2003.

17. Mackenzie, P. I. Expression of chimeric cDNAs in cell culture defines a region of UDP-glucuronosyltransferase involved in substrate selection. J. Biol. Chem., *265:* 3432-3435, 1990.

18. Miners, J. O., Smith, P. A., Sorich, M. J., McKinnon, R. A., and Mackenzie, P. I. Predicting human drug glucuronidation parameters: Application of in vitro and in silico modeling approaches. Annu. Rev. Pharmacol. Toxicol., *44:* 1-25, 2004.

19. Tukey, R. H. and Strassburg, C. P. Human UDP-glucuronosyltransferases: Metabolism, Expression, Disease. Annu. Rev. Pharmacol. Toxicol., *40:* 581-616, 2000.

20. Munzel, P. A., Schmohl, S., Heel, H., Kålberer, K., Bock-Hennig, B. S., and Bock, K. W. Induction of human UDP-glucuronosyltransferases (UGT1A6, UGT1A9 and UGT2B7) by *t*-butylhydroquinone and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in Caco-2 cells. Drug Metabolism and Disposition, *27:* 569-573, 1999.

21. Yueh, M.-F., Huang, Y. H., Hiller, A., Chen, S., Nguyen, N., and Tukey, R. H. Involvement of the xenobiotic response element (XRE) in Ah receptor-mediated

induction of human UDP-glucuronosyltransferase 1A1. The Journal of Biological Chemistry, *278:* 15001-15006, 2003.

22.   Bock, K. W., Eckle, T., Ouzzine, M., and Fournel-Gigleux, S. Coordinate induction by antioxidants of UDP-glucuronosyltransferase UGT1A6 andthe apical conjugate export pump MRP2 (multidrug resistance protein 2) in Caco-2 cells. Biochemical Pharmacology, *59:* 467-470, 2000.

23.   Ritter, J. K., Kessler, F., Thompson, M. T., Grove, A. D., Auyeung, D. J., and Fisher, R. A. Expression and inducibility of the human bilirubin UDP-glucuronosyltransferase UGT1A1 in liver and cultured primary hepatocytes: evidence for both genetic and environmental influences. Hepatology, *30:* 476-484, 1999.

24.   Walle, U. and Walle, T. Induction of UDP-glucuronosyltransferase UGT1A1 by flavonoids-structural requirements. Drug Metabolism and Disposition, *30:* 564-569, 2002.

25.   van der Logt, E. M. J., Roelofs, H. M. J., Nagengast, F. M., and Peters, W. H. M. Induction of rat hepatic and intestinal UDP-glucurononsyltransferase by naturally occurring dietary anticarcinogens. Carcinogenesis, *24:* 1651-1656, 2003.

26.   Sugatani, J., Kojima, H., Ueda, A., Kakizaki, S., Yoshinari, K., Gong, Q. H., Owens, I. S., Negishi, M., and Sueyoshi, T. The phenobarbital response enhancer module in the human bilirubin UDP-glucuronosyltransferase UGT1A1 gene and regulation by the nuclear receptor CAR. Hepatology, *33:* 1232-1238, 2001.

27.   Xie, W., Yeuh, M. F., Radominska-Pandya, A., Saini, S. P., Negishi, Y., Bottroff, B. S., Cabrera, G. Y., Tukey, R. H., and Evans, R. M. Control of steroid, heme, and carcinogen metabolism by nuclear pregnane X receptor and constitutive androstane receptor. Proceedings of the National Acadamy of Sciences of the United States ofAmerica, *100:* 4150-4155, 2003.

28.   Duvaldestin, P., Mahu, J. L., Preaux, A. M., and Berthelot, P. Novobiocin-inhibition and magnesium-interaction of rat liver microsomal bilirubin UDP-glucuronosyltransferase. Biochemical Pharmacology, *25:* 2587-2592, 1976.

29.   Krcmery, M. and Zakim, D. Effects of oleoyl-CoA on the activity and functional state of UDP-glucuronosyltransferase. Biochem. Parmacol., *46:* 897-904, 1993.

30.   Csala, M., Banhegyi, G., Kardon, T., Fulceri, R., Gamberucci, A., Giunti, R., A., B., and Mandl, J. Inhibition of glucuronidation by an Acyl-CoA-mediated indirect mechanism. Biochemical Pharmacology, *52:* 1127-1131, 1996.

31. Strassburg, C. P., Manns, M. P., and Tukey, R. H. Differential down-regulation of the UDP-glucuronosyltransferase 1A locus is an early event in human liver and bilary cancer. Cancer Research, *57:* 2979-2985, 1997.

32. Bhasker, C. R., McKinnon, W., Stone, A., Lo, A. C., Kubota, T., Ishizaki, T., and Miners, J. O. Genetic polymorphisms of UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significicnace. Pharmacogenetics, *10:* 670-685, 2000.

33. Tukey, R. H. and Strassburg, C. P. Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. Molecular Pharmacology, *59:* 405-414, 2001.

34. Strassburg, C. P., Kneip, S., Topp, J., Obermayer-Straub, P., Barut, A., Tukey, R. H., and Manns, M. P. Polymorphic gene regulation and interindividual variation of UDP-glucuronosyltransferase activity in human small intestine. J. Biol. Chem., *275:* 36164-36171, 2000.

35. Minors, J. O., McKinnon, R. A., and Mackenzie, P. I. Genetic polymorphisms of UDP-glucuronosyltransferase and their functional significance. Toxicology, *181-182:* 453-456, 2002.

36. Grant, D. J. and Bell, D. A. Bilirubin UDP-glucuronosyltransferase 1A1 gene polymorphisms: Susceptibility to oxidative damage and cancer. Molecular Carcinogenesis, *29:* 198-204, 2000.

37. Strassburg, C. P., Vogel, A., Kneip, S., Tukey, R. H., and Manns, M. P. Polymorphisms of the human UDP-glucuronosyltransferase (UGT) 1A7 gene in colorectal cancer. Gut, *50:* 851-856, 2002.

38. Ockenga, J., Vogel, A., Teich, N., Keim, V., Manns, M. P., and Strassburg, C. P. UDP-glucuronosyltransferase (UGT1A7) gene polymorphisms increase the risk of chronic pancreatitis and pancreatic cancer. Gastroenterology, *124:* 1802-1808, 2003.

39. Burchell, B. Genetic variation of human UDP-glucuronosyltransferase: implications in disease and drug glucuronidation. American Journal of Pharmacogenomics, *3:* 37-52, 2003.

40. Adegoke, O. J., Shu, X. O., Gao, Y. T., Cai, Q., Breyer, J., Smith, J., and Zheng, W. Genetic polymorphisms in uridine diphospho-glucuronosyltransferase 1A1 (UGT1A1) and risk of breast cancer. Breast Cancer Research and Treatment, *85:* 239-245, 2004.

41.   Ritter, J. K., Crawford, J. M., and Owens, I. S. Cloning of two human liver bilirubin UDP-glucuronosyltransferase cDNAs with expression in COS-1 cells. J. Biol Chem, *266:* 1043-1047, 1991.

42.   Bosma, P. J., Seppen, J., Goldhoorn, B., Bakker, C., Oude Elferink, R. P., Roy-Chowdhury, J., Roy-Chowdhury, N., and Jansen, P. L. Bilirubin UDP-glucuronosyltransferase 1 is the only relevant bilirubin glucuronidating isoform in man. J. Biol. Chem., *269:* 17960-17964, 1994.

43.   Bosma, P. J., Chowdhury, J. R., Bakker, C., Gantla, S., deBoer, A., Oostra, B. A., Lindhout, D., Tygat, G. N., Jansen, P. L., Oude Elferink, R. P., and Chowdury, N. R. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. New England Journal of Medicine, *333:* 1171-1175, 1995.

44.   Rauchschwalbe, S. K., Zuhlsdorf, M. T., Schuhly, U., and Kuhlman, J. Predicting the risk of sporadic elevated bilirubin levels and diagnosing Gilbert's syndrome by genotyping UGT1A1*28 promoter polymorphism. Int. J. Clin. Pharmacol. Ther., *40:* 233-240, 2002.

45.   Mackenzie, P. I., Miners, J. O., and McKinnon, R. A. Polymorphisms in UDP-glucuronosyltransferase Genes: Functional consequences and clinical relevance. Clin. Chem. Lab. Med., *38:* 889-892, 2000.

46.   Fang, J.-L. and Lazarus, P. Correlation between the UDP-glucuronosyltransferase (UGT1A1) TATAA box polymorphism and carcinogen detoxification phenotype: Significantly decreased glucuronidating activity against benzo(a)pyrene-7,8-dihydrodiol(—) in liver microsomes from subjects with the UGT1A1*28 variant. Cancer Epidemiology, Biomarkers and Prevention, *13:* 102-109, 2004.

47.   Orzechowski, A., Schrenk, D., Bock-Hennig, B. S., and Bock, K. W. Glucuronidation of carcinogenic arylamines and their N-hydroxy derivatives by rat and human phenol UDP-glucuronosyltransferases of the UGT1 gene complex. Carcinogenesis, *15:* 1549-1553, 1994.

48.   Ritter, J. K., Sheen, Y. Y., and Owens, I. S. Cloning and expression of human liver glucuronosyltransferase in COS-1 cells. 3,4-catechol estrogens and estriol as primary substrates. Journal of Biological Chemistry, *265:* 7900-7906, 1990.

49.   Coffman, B. L., King, C. D., Rios, G. R., and Tephly, T. R. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). Drug Metabolism and Disposition, *26:* 73-77, 1998.

50.   Levesque, E., Beaulieu, M., Green, M. D., Tephly, T. R., Belanger, A., and Hum, D. W. Isolation and characterization of UGT2B15(Y85): a UDP-

glucuronosyltransferase encoded by a polymorphic gene. Pharmacogenetics, *7:* 317-325, 1997.

51.   Hu, Z. and Wells, P. G. In vitro and in vivo biotransformation and covalent binding of benzo(a)pyrene in Gunn and RHA rats with a genetic deficiency in bilirubin uridine diphosphate-glucuronosyltransferase. The Journal of Pharmacology and Experimental Therapeutics, *263:* 334-342, 1992.

52.   Hu, Z. and Wells, P. G. Human interindividual variation in lymphocyte UDP-glucuronosyltransferases as a determinant of in vitro benzo[a]pyrene covalent binding and cytotoxicity. Toxicological Sciences, *78:* 32-40, 2004.

53.   Sugimura, T. and Sato, S. Mutagens-carcinogens in foods. Cancer Res. Suppl., *43:* 2415s-2421s, 1983.

54.   Wakabayashi, K., Nagao, M., Esumi, H., and Sugimura, T. Food-derived mutagens and carcinogens. Cancer Res. (suppl.), *52:* 2092s-2098s, 1992.

55.   Adamson, R. H., Gustafsson, J.-Å., Ito, N., Nagao, M., Sugimura, T., Wakabayashi, K., and Yamazoe, Y. (eds.) Heterocyclic amines in cooked foods: Possible human carcinogens. Princeton, New Jersey: Princeton Scientific Publishing Co., Inc., 1995.

56.   Miller, E. C. and Miller, J. Carcinogens and mutagens that may occur in foods. Cancer, *58:* 1795-1803, 1986.

57.   Sugimura, T. and Wakabayashi, K. Mutagens and Carcinogens in Foods. *In:* M. W. Pariza (ed.), Mutagens and Carcinogens in the Diet., pp. 1-18. New York: Wiley-Liss, 1990.

58.   Widmark, E. M. P. Presence of cancer-producing substances in roasted food. Nature, *143:* 984, 1939.

59.   Nagao, M., Honda, M., Seino, Y., Yahagi, T., and Sugimura, T. Mutagenicities of smoke condensates and the charred surface of fish and meats. Cancer Letters, *2:* 221-226, 1977.

60.   Commoner, B., Vithayathil, A., Dolara, P., Nair, S., Madyastha, P., and G., C. Formation of mutagens in beef and beef extracts during cooking. Science, *201:* 913-916, 1978.

61.   Felton, J. S., Knize, M. G., Shen, N. H., Andresen, B. D., Bjeldanes, L. F., and Hatch, F. T. Identification of the mutagens in cooked beef. Environ. Health Perspect., *67:* 17-24, 1986.

62. Sugimura, T. Overview of carcinogenic heterocyclic amines. Mutation Research, *376:* 211-219, 1997.

63. Turteltaub, K. W., Dingley, K. H., Curtis, K. D., Malfatti, M. A., Turesky, R. J., Garner, R. C., Felton, J. S., and Lang, N. P. Macromolecular adduct formation and metabolism of heterocyclic amines in humans and rodents at low doses. Cancer Letters, *143:* 149-156, 1999.

64. Mauthe, R. J., Dingley, K. H., Leveson, S. h., Freeman, S. P., Turesky, R. J., Garner, R. C., and Turteltaub, K. W. Comparison of DNA-adduct and tissue-available dose levels of MeIQx in human and rodent colon following adminstration of a very low dose. International Journal of Cancer Research, *80:* 539-545, 1999.

65. Garner, R. C., Lightfoot, T. J., Cupid, B. C., Russell, D., Coxhead, J. M., Kutschera, W., Priller, A., Rom, W., Steier, P., Alexander, D. J., Leveson, S. H., Dingley, K. H., Mauthe, R. J., and Turteltaub, K. W. Comparative biotransformation studies of MeIQx and PhIP in animal models and humans. Cancer Letters, *143:* 161-165, 1999.

66. Sinha, R., Kulldorff, M., Chow, W. H., Denobile, J., and Rothman, N. Dietary intake of heterocyclic amines, meat-derived mutagenic activity, and risk of colorectal adenomas. Cancer Epidemiology, Biomarkers and Prevention, *10:* 559-562, 2001.

67. Felton, J. S., Knize, M. G., Shen, N. H., Lewis, P. R., Anderson, B. D., Happe, J., and Hatch, F. T. The isolation and identification of a new mutagen from fried ground beef: 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP). Carcinogenesis, *7:* 1081-1086, 1986.

68. Esumi, H., Ohgaki, H., Kohzen, E., Takayama, S., and Sugimura, T. Induction of Lymphoma in CDF1 Mice by the Food Mutagen, 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. Jpn. J. Cancer Res., Gann., *80:* 1176-1178, 1989.

69. Dooley, K. L., Von Tungeln, L. S., Bucci, T., Fu, P. P., and Kadlubar, F. F. Comparative carcinogenecity of 4-aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F$_1$ male mouse. Cancer Letters, *62:* 205-209, 1992.

70. Thompson, L. H., Tucker, J. D., Stewart, S. A., Christensen, M. L., Salazar, E. P., Carrano, A. V., and Felton, J. S. Genotoxicity of compounds from cooked beef in repair-deficient CHO cells versus *Salmonella* mutagenicity. Mutagenesis, *2:* 483-487, 1987.

71. Buonarati, M. H., Tucker, J. D., Minkler, J. L., Wu, R. W., Thompson, L. H., and Felton, J. S. Metabolic activation and cytogenetic effects of 2-amino-1-methyl-6-

phenylimidazo[4,5-*b*]pyridine (PhIP) in Chinese hamster overy cells expressing murine cytochrome P4501A2. Mutagenesis, *6:* 253-259, 1991.

72.   Buonarati, M. H., Turteltaub, K. W., Shen, N. H., and Felton, J. S. Role of sulfation and acetylation in the activation of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine to intermediates which bind DNA. Mutation Research, *245:* 185-190, 1990.

73.   Dragsted, L. O., Frandsen, H., Reistad, R., Alexander, J., and Larsen, J. C. DNA-binding and disposition of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in the rat. Carcinogenesis, *16:* 2785-2793, 1995.

74.   Fan, L., Schut, H. A. J., and Snyderwine, E., G. Cytotoxicity, DNA adduct formation and DNA repair induced by 2-hydroxyamino-3-methylimidazo[4,5-*f*]quinoline and 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in cultered human mammary epithelial cells. Carcinogenesis, *16:* 775-779, 1995.

75.   Dingley, K. H., Curtis, K. D., Nowell, S., Felton, J. S., Lang, N. P., and Turteltaub, K. W. 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 Epidemiology, Biomarkers & Prevention, *8:* 507-512, 1999.

76.   Zheng, W., Gustafson, D. R., Sinha, R., Cerhan, J. R., Moore, D., Hong, C. P., Anderson, K. E., Kushi, L. H., Sellers, T. A., and Folsom, A. R. Well-done meat intake and the risk of breast cancer. J. Natl. Cancer Inst., *90:* 1724-1729, 1998.

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

78.   Kato, R. and Yamazoe, Y. Metabolic activation and covalent binding to nucleic acids of carcinogenic heterocyclic amines from cooked foods and amino acid pyrolysates. Japan Journal of Cancer Research, *78:* 297-311, 1987.

79.   Ozawa, S., Chou, H.-C., Kadlubar, F. F., Nagata, K., Yamazoe, Y., and Kato, R. Activation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by cDNA-expressed human and rat arylsulfotransferases. Japan Journal of Cancer Research, *85:* 1220-1228, 1994.

80.   Davis, C. D., Schut, H. A. J., and Snyderwine, E. G. Enzymatic phase II activation of the N-hydroxylamines of IQ, MeIQx and PhIP by various organs of monkeys and rats. Carcinogenesis, *14:* 2091-2096, 1993.

81.   Alexander, J., Wallin, H., Rossland, O. J., Solberg, K., Holme, J. A., Becher, G., Andersson, R., and Grivas, S. Formation of a glutathione conjugate and a

semistable transportable glucuronide conjugate of N2-oxidized species of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in rat liver. Carcinogenesis, *12:* 2239-2245, 1991.

82.   Kaderlik, K. R., Mulder, G. J., Turesky, R. J., Lang, N. P., Teitel, C. H., Chiarelli, M. P., and Kadlubar, F. F. Glucuronidation of N-hydroxy heterocyclic amines by human and rat liver microsomes. Carcinogenesis, *15:* 1695-1701, 1994.

83.   Buonarati, M., Roper, M., Morris, C., Happe, J., Knize, M., and Felton, J. Metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in mice. Carcinogenesis, *13:* 621-627, 1992.

84.   Watkins, B. E., Suzuki, M., Wallin, H., Wakabayashi, K., Alexander, J., Vanderlaan, M., Sugimura, T., and Esumi, H. The effect of dose and enzyme inducers on the metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in rats. Carcinogenesis, *12:* 2291-2295, 1991.

85.   Murray, B. P., Edwards, R. J., Murray, S., Singleton, A. M., Davies, D. S., and Boobis, A. R. Human hepatic CYP1A1and CYP1A2 content, determined with specific anti-peptide antibodies, correlates with the mutagenic activation of PhIP. Carcinogenesis, *14:* 585592, 1993.

86.   Hammons, G. J., Milton, D., Stepps, K., Guengerich, F. P., Tukey, R. H., and Kadlubar, F. F. Metabolism of carcinogenic heterocyclic and aromatic amines by recombinant human cytochrome P450 enzymes. Carcinogenesis, *18:* 851-854, 1997.

87.   Styczynski, P. B., Blackmon, R. C., Groopman, J. D., and Kensler, T. W. The direct glucuronidation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) in human and rabbit liver microsomes. Chemical Research in Toxicology, *6:* 846-851, 1993.

88.   Malfatti, M. A., Kulp, K. S., Knize, M. G., Davis, C., Massengill, J. P., Williams, S., Nowell, S., MacLeod, S., Dingley, K. H., Turteltaub, K. W., Lang, N. P., and Felton, J. S. The identification of [2-^{14}C]2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine metabolites in humans. Carcinogenesis, *20:* 705-713, 1999.

89.   Kulp, K. S., Knize, M. G., Malfatti, M. A., Salmon, C. P., and 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, *21:* 2065-2072, 2000.

90.   Nowell, S. A., Massengill, J. S., Williams, S., Radominska-Pandya, A., Tephly, T. R., Cheng, Z., Strassburg, C. P., Tukey, R. H., MacLeod, S. L., Lang, N. P., and Kadlubar, F. F. 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, *20:* 1107-1114, 1999.

91.   Yueh, M.-F., Nguyen, N., Famourzadeh, M., Strassburg, C. P., Oda, Y., Guengerich, P. F., and Tukey, R. H. The contribution of UDP-glucuronosyltransferase 1A9 on CYP1A2-mediated genotoxicity by aromatic and heterocyclic amines. Carcinogenesis, *22:* 943-950, 2001.

92.   Malfatti, M. A. and 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, *22:* 1087-1093, 2001.

93.   Malfatti, M. A. and Felton, J. S. Human UDP-glucuronosyltransferase 1A1 is the primary enzyme responsible for the N-glucuronidation of N-hydroxy-PhIP in vitro. Chemical Research in Toxicology, *17:* 1137-1144, 2004.

94.   Kadlubar, F. F., Butler, M. A., Kaderlik, K. R., Chou, H.-C., and Lang, N. P. Polymorphisms for aromatic amine metabolism in humans: relevance for human carcinogenesis. Environmental Health Perspectives, *98:* 69-74, 1992.

95.   Turesky, R. J. Heterocyclic aromatic amine metabolism, DNA adduct formation, mutagenesis, and carcinogenesis. Drug Metabolism and Disposition, *34:* 625-650, 2002.

96.   Malfatti, M. A., Ubick, E. A., and Felton, J. S. The Impact of glucuronidation on the bioactivation of the cooked-food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine *in vivo*. submitted, 2005.

97.   Dietrich, C. G., Ottenhoff, R., de Waart, D. R., and Oude Elferink, R. P. J. Lack of UGT1 isoform in Gunn rats changes metabolic ratio and facilitates excretion of the food-derived carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine. Toxicology and Applied Pharmacology, *170:* 137-143, 2001.

98.   Malfatti, M. A., Wu, R. W., and Felton, J. S. The effect of UDP-glucuronosyltransferase 1A1 expression on the mutagenicity and metabolism of the cooked-food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in CHO cells. Mutation Research, *570:* 205-214, 2005.

Figure Legends

Figure 1. Organization of the UGT1A gene locus, and an example of how different UGT1A RNAs are processed. Exons 2-5 are common to all UGT1A isoforms. Exon 1 contains sequences that code for the divergent portion of each UGT1A protein, represented by exons 1.1-1.13. Transcription is initiated at promoters that flank each of the exon-1 sequences. The 5' and 3' consensus-splice sites are recognized by the spliceosome and the intervening sequences are removed *(Figure was adapted from Tukey and Strassburg, 2000, Annu. Rev. Toxicol., 40, 581-616).*

Figure 2. Carcinogenic heterocyclic amines isolated from cooked foods. PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; DMIP, 2-amino-1,6-dimethylimidazo[4,5-*b*]pyridine ; MeIQ, 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; 4,8-DiMeIQx, 2-amino-3,4,8-trimethylimidazo[4,5-*f*]quinoxaline.

Figure 3. Overview of PhIP metabolism

Figure 4. Species comparison of urinary PhIP metabolic profiles. A: human; B: dog; C: mouse.
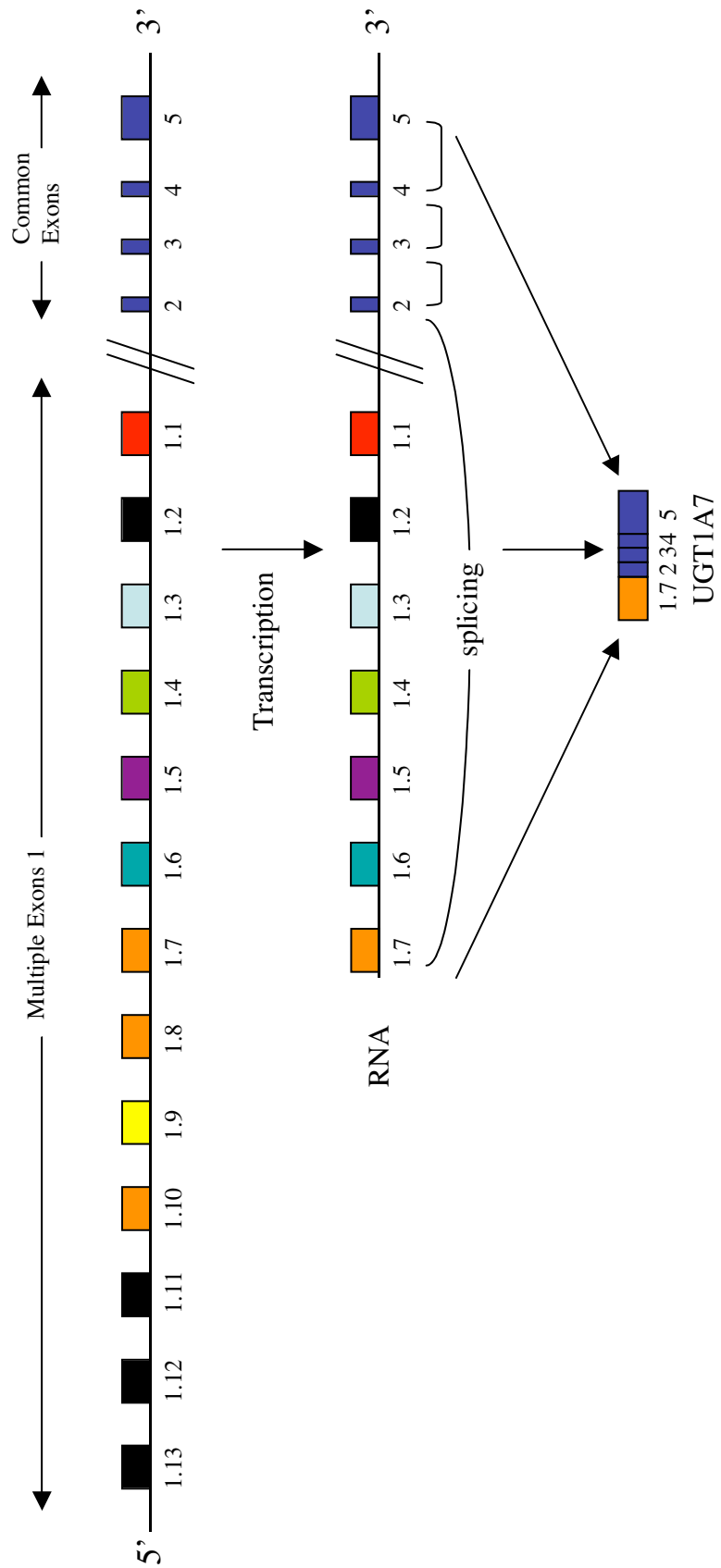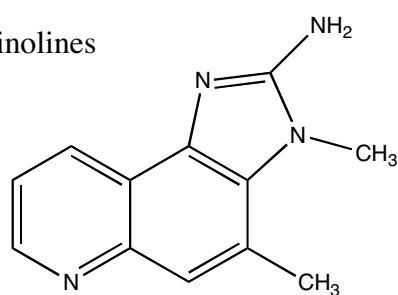
Figure 1

Pyridines
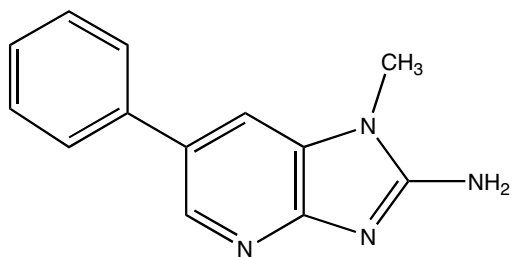
PhIP
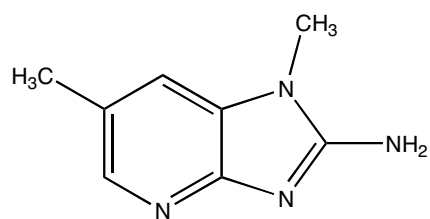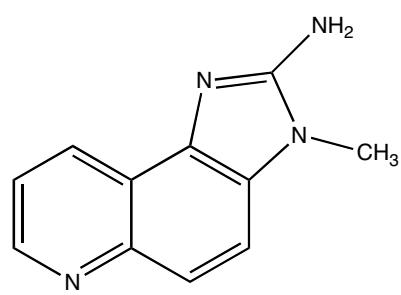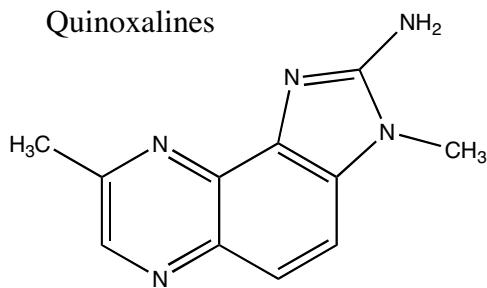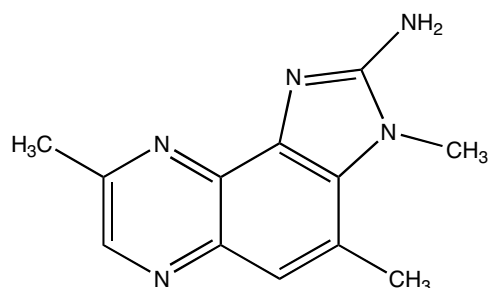
DMIP

Quinolines

MeIQ

IQ

Quinoxalines

MeIQx

4,8-DiMeIQx

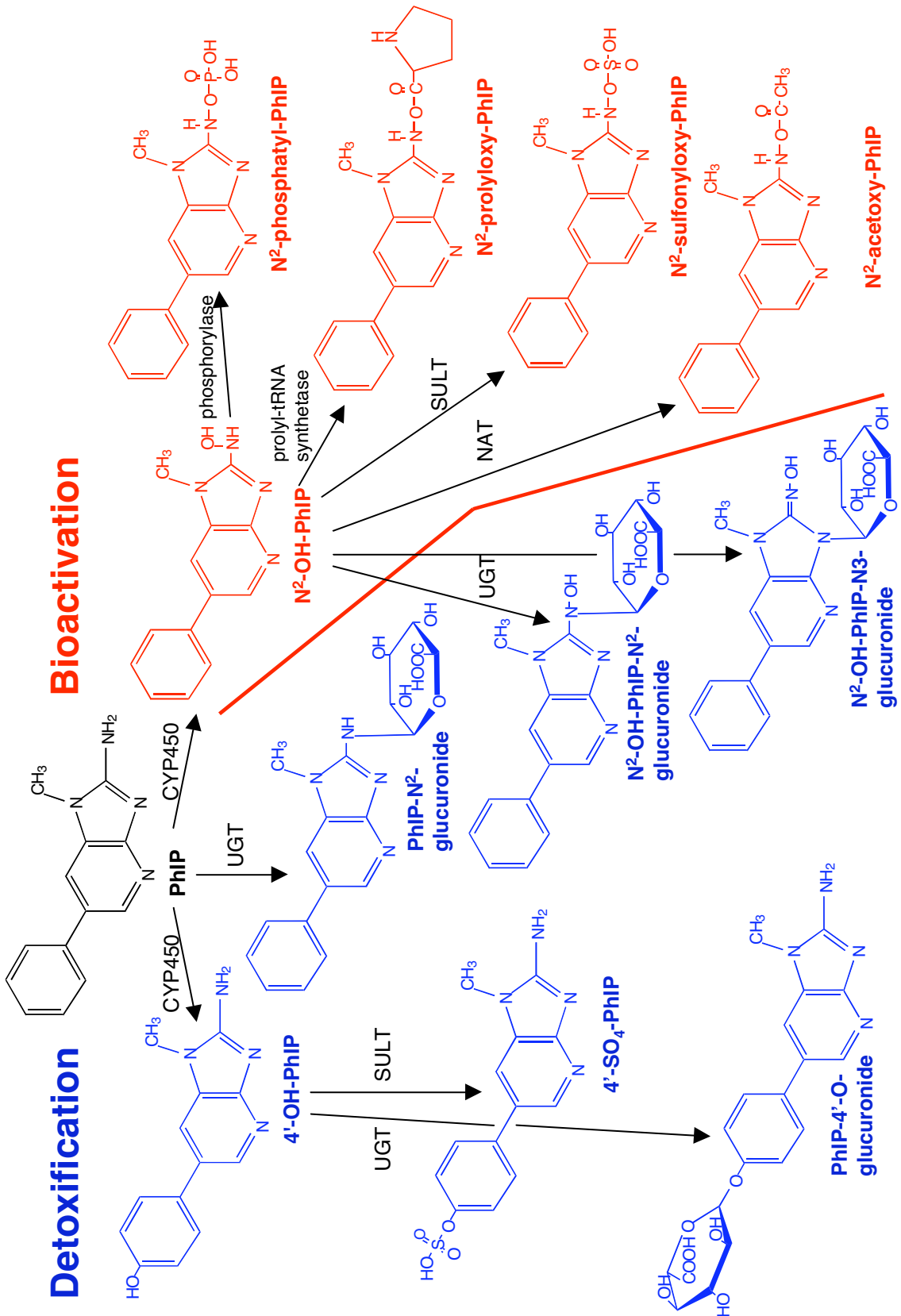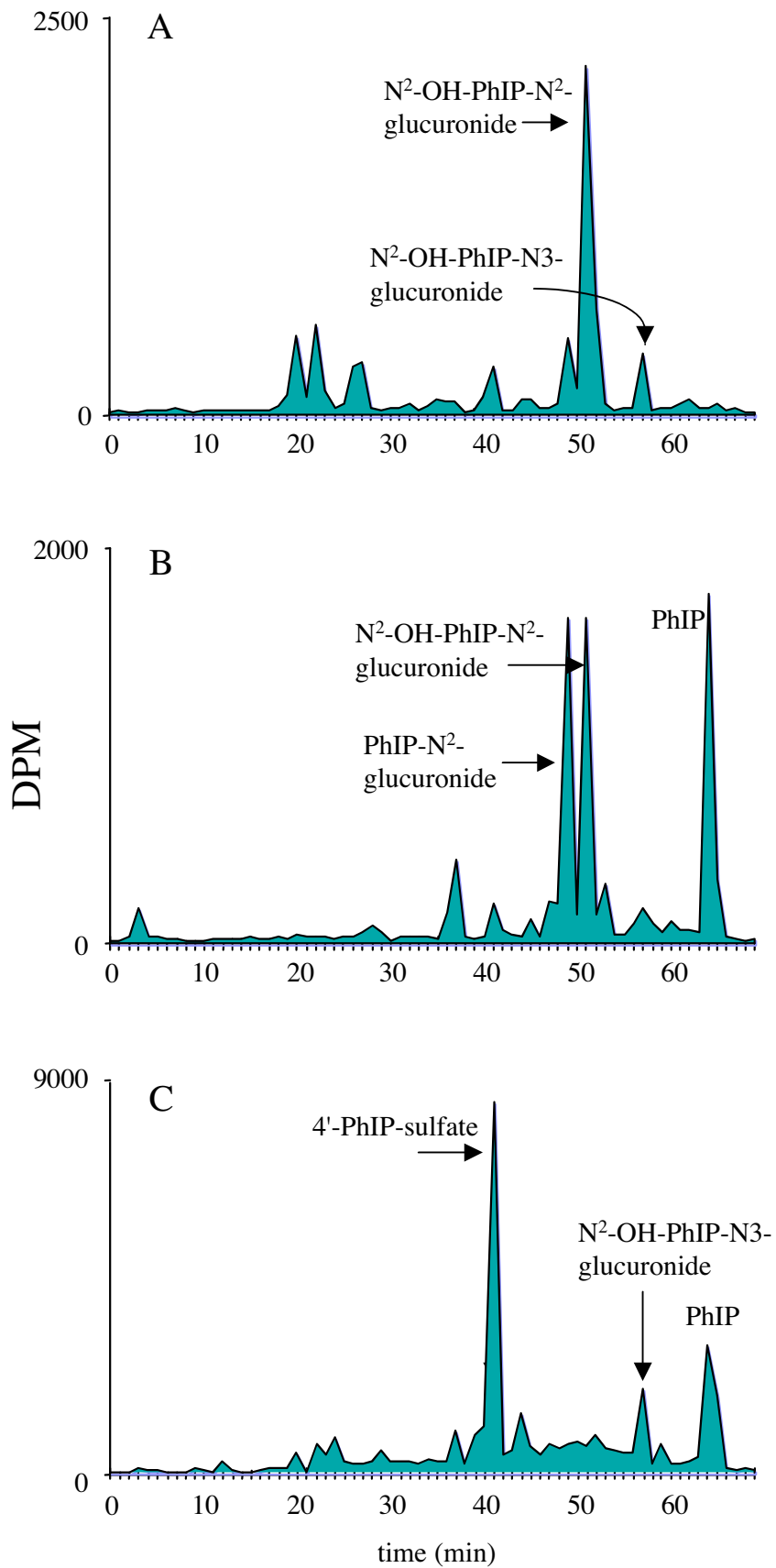Figure 2

Figure 3

Figure 4