CYTOCHROME P450 ACTIVATION OF ARYLAMINES
AND HETEROCYCLIC AMINES

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Abstract  Arylamines and heterocyclic arylamines (HAAs) are of particular interest because of demonstrated carcinogenicity in animals and humans and the broad exposure to many of these compounds. The activation of these, and also some arylamine drugs, involves N-hydroxylation, usually by cytochrome P450 (P450). P450 1A2 plays a prominent role in these reactions. However, P450 1A1 and 1B1 and other P450s are also important in humans as well as experimental animals. Some arylamines (including drugs) are N-hydroxylated predominantly by P450s other than those in Family 1. Other oxygenases can also have roles. An important issue is extrapolation between species in predicting cancer risks, as shown by the low rates of HAA activation by rat P450 1A2 and low levels of P450 1A2 expression in some nonhuman primates.

INTRODUCTION

Many arylamines, e.g., 2-naphthylamine (2-NA), benzidine, and 4-aminobiphenyl (4-ABP), are of industrial importance because of their use as intermediates in the synthesis of azo dyes, antioxidants in rubber products, and other commercial materials (1, 2). Epidemiological observations of the toxicity of arylamines were first reported in aniline dye factories by Rehn in 1895, with the report that German and Swiss workers suffered urinary bladder tumors (2, 3). A major toxicological issue is reaction with DNA and induction of carcinomas, primarily in the urinary bladder, liver, or other tissues in humans and experimental animals (1, 2, 4–6).

In 1939, the Swedish chemist Widmark demonstrated that extracts of fried horse meat induced cancer when applied to mouse skin (7, 8). Sugimura and his associates investigated the smoke produced by broiling fish and meat; they demonstrated that the smoke condensate and charred surfaces of broiled fish and meat were highly mutagenic in Salmonella typhimurium test systems (Table 1) (9–11). Subsequently, the heterocyclic arylamine (HAA) products formed as a consequence of pyrolysis of amino acids or protein-containing foods were isolated, their structures were...
KIM ■ GUENGERICH

TABLE 1  Mutagenicity of HAAs in *S. typhimurium* tester strains

<table>
<thead>
<tr>
<th>HAA</th>
<th>Revertants/µg HAA</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-amino-3-methylimidazo[4,5-f]quinoline (IQ)</td>
<td>433,000</td>
</tr>
<tr>
<td>2-amino-3,5-dimethylimidazo[4,5-f]quinoline (MeIQ)</td>
<td>661,000</td>
</tr>
<tr>
<td>2-amino-3-methylimidazo[4,5-f]quinoline (IQx)</td>
<td>75,000</td>
</tr>
<tr>
<td>2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx)</td>
<td>145,000</td>
</tr>
<tr>
<td>2-amino-3,4,8-trimethylimidazo[4,5-f]quinoxaline (4,8-1)</td>
<td>183,000</td>
</tr>
<tr>
<td>2-amino-3,7,8-trimethylimidazo[4,5-f]quinoxaline (1,7-1)</td>
<td>163,000</td>
</tr>
<tr>
<td>2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)</td>
<td>1800</td>
</tr>
<tr>
<td>3-amino-1,4-dimethyl-5H-pyrido[4,3-b]-indole (Trp-P-1)</td>
<td>39,000</td>
</tr>
<tr>
<td>3-amino-1-methyl-5H-pyrido[4,3-b]-indole (Trp-P-2)</td>
<td>104,000</td>
</tr>
<tr>
<td>2-amino-6-methyldipyrido[1,2-a:3',2'-d]imidazole (Glu-P-1)</td>
<td>49,000</td>
</tr>
<tr>
<td>2-aminodipyrido[1,2-a:3',2'-d]imidazole (Glu-P-2)</td>
<td>1900</td>
</tr>
<tr>
<td>2-amino-5-phenylpyridine (Phe-P-1)</td>
<td>41</td>
</tr>
<tr>
<td>2-amino-9H-pyrido[2,3-b]indole (AαC)</td>
<td>56,800</td>
</tr>
</tbody>
</table>

*aReference 11.

determined, and their biological effects were examined, specifically mutagenicity and carcinogenicity in animals (12–16).

The formation of HAAs is the result of a Maillard reaction (8, 17–20). This reaction occurs when amino acids (proteins) and reducing sugars (carbohydrates) are heated together. More than 20 HAAs have been identified (Figure 1) (8, 14, 21–23). HAAs were also identified in cigarette smoke condensate and shown to be genotoxic (24, 25). Several antimicrobial drugs contain arylamine moieties, e.g., sulfamethoxazole (SMX) and dapsone. Potential roles of *N*-hydroxy arylamine metabolites in mediating the idiosyncratic reactions and the importance of oxidative metabolism in their toxicities have been investigated (26, 27). Arylamines are also formed in commercial hair dyes, and their contributions to an increased risk of bladder, breast, colon, and lymphatic cancer have been investigated (28–30).

CHEMISTRY OF BIOACTIVATION

Arylamines and HAAs require metabolic activation to be mutagenic or carcinogenic (31). The major metabolic process is *N*-oxidation, which is mediated primarily by cytochrome P450 (P450) enzymes but also by flavin-containing monooxygenases (FMOs) and peroxidases (31–39). The resulting *N*-hydroxylamine products can be further activated to produce highly reactive ester derivatives that
Figure 1  Some of the HAAs found in food.
bind covalently to DNA. At least four enzyme systems are known to be involved in this secondary activation step in mammals: \(N\)-acetyltransferase (NAT), sulfotransferase, prolyl tRNA synthetase, and kinases, yielding reactive \(N\)-acetoxy, \(N\)-sulfonloxy, \(N\)-prolyloxy, and \(N\)-phosphatyl esters, respectively (40–43). The \(N\)-hydroxy HAAs can react directly with DNA (32), but the reaction is facilitated when reactive ester derivatives undergo heterocyclic cleavage to yield reactive aryl nitrenium ion species, which preferentially react to form DNA adducts (Figure 2). NAT-catalyzed acetylation of \(N\)-hydroxy HAAs and arylamines enhances genotoxic activity and DNA adduct levels through formation of reactive \(N\)-acetoxy esters (44–48). In a similar way, the sulfur esters formed by the action of sulfotransferases are unstable and react (42, 49). The role of the sulfotransferases has been given less attention than NAT in human epidemiology studies. Even less information is available about the in vivo roles of the prolyloxy and phosphatyl esters.

Arylamines and HAAs yield adducts primarily with guanine (50, 51), reacting at the N2 and C8 atoms. Wild et al. (52) showed that the photoactivated azide derivatives of IQ, MeIQx, and PhIP bind to DNA to form the same adducts as the \(N\)-acetoxy species, indicating that the nitrenium ion may be a common intermediate for both reactive intermediates. Mechanisms for the reaction at the C8 atom have been less clear (53). A direct reaction is possible (54), and a stepwise mechanism via an \(N^7\)-guanyl intermediate has also been proposed (55, 56). The latter has an advantage of also explaining several accompanying products (e.g., 8-oxo-7,8-dihydroguanine, depurination, imidazole ring opening) (56).

Several approaches to syntheses of DNA adducts of these amines have been reported. HAA-DNA adducts (including IQ, MeIQ, MeIQx, 4,8-DiMeIQx, PhIP, Glu-P-1, and Trp-P-2) have been synthesized and characterized spectroscopically (57–63). Oligonucleotides containing guanyl-C8 2-aminofluorene (2-AF) (and \(N\)-acetyl 2-AF) derivatives have been synthesized, and structures have been determined using NMR spectroscopy and mutagenic properties have been examined in cell-based systems (Figure 3). PhIP has been site-specifically incorporated into oligonucleotides by a biomimetic approach in which \(N\)-acetoxy-PhIP was reacted with oligonucleotides containing a single guanine (64). In a nonbiomimetic approach, the Rizzo group has reported the synthesis of C8-guanyl adduct of IQ through palladium-catalyzed \(N\)-arylation of a protected 8-bromo-2′-dG derivative with IQ as the key reaction (65).

SYSTEMS FOR ANALYSIS OF MUTATION AND CANCER

Arylamines have long been known to be mutagenic following metabolic activation (66), and an early study showed the mutagenicity of hair dyes (67). HAAs have shown strong mutagenicity in \(S.\ typhimurium\) strains since their discovery (9, 10, 68). HAAs preferentially induce the frameshift mutations in CG repeat of the \(hisD^+\) gene, as opposed to causing base pair mutations (69, 70). This type of
Figure 2  General pathway for activation of arylamines and HAAs, as shown for IQ.
Figure 3  Structures of some carcinogenic arylamines.

mutation response is also found in other bacterial genes, such as lacZ, lacZα, and lacI of Escherichia coli (71, 72). An E. coli lacZ reversion mutation assay has also been applied to study HAA genotoxicity (73–76); a tester strain carrying a (-GC) copy of lacZ gene can regain functional lacZ activity following induction of frameshifts by HAAs (76, 77). Systems have also been developed that incorporate the heterologous expression of P450s and NADPH-P450 reductase (74). This system allows the detection of HAA mutagenicity by recombinant human P450 without a need for rat liver fractions. These bacteria have also been genetically engineered to express S. typhimurium NAT, and the DNA nucleotide excision repair system has been inactivated (UvrABC) to improve the sensitivity (74, 77, 78). S. typhimurium tester strains have also been used to express human P450s that activate arylamines and HAAs (73, 79, 80). The E. coli strains overexpressing P450s and NAT have been used to characterize P450 1A2 allelic and random variants (81–84). Another use of this genotoxicity system has been the screening and characterization of P450 inhibitors. For instance, a P450 1B1–based system was used to characterize the potent inhibition of the enzyme by tetramethoxystibene (85) and a P450 1A2–based system was sensitive to the drug oltipraz (86). Other bacterial systems have utilized the SOS response in S. typhimurium NM2009 as a measure of DNA damage (47). This strain contains a plasmid-based umuC gene linked to a lacZ reporter gene and is activated by induction of the SOS pathway (47). Sensitivity to aryldamines and HAAs was also improved by incorporating plasmids coding for P450 enzymes, NADPH-P450 reductase, and NAT (48).

DNA adducts are considered biomarkers of potential mutagenesis and carcinogenesis (87, 88), and HAAs are thought to induce mutagenesis by producing mutations in oncogenes and tumor suppressor genes in experimental animals (23, 89). Although base pair mutations are not a major feature of HAAs in bacterial test systems, one was dominantly induced by IQ in the p53 gene in rat Zymbal’s gland tumors and monkey hepatocellular carcinoma (89, 90). Also, a C → T transversion
in ras was predominantly observed in mouse forestomach and rat Zymbal’s gland tumors induced by MeIQ. However, PhIP was reported to induce a number of characteristic one-base deletions in the lacI gene of the colon mucosa of transgenic mice (91).

The major analytical methods used for the detection and quantification of arylamine and HAA adducts are the ³²P-postlabeling assay and mass spectrometry. ³²P-postlabeling assays use polynucleotide kinase to label the adducted or nonadducted ³′-nucleotides with [γ-³²P]ATP after digestion of DNA to mononucleotides (92). The radiolabeled ³′, ⁵′-bisphosphonucleotide adducts can be separated by two-dimensional thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) (93). A combination of HPLC and electrospray ionization-tandem mass spectrometry can provide structural information on adducts, and the incorporation of stable isotopically labeled internal standards in assays provides precise and accurate quantification of the DNA adducts (93).

N-Hydroxylamines and other metabolites of arylamines and HAAs are mainly identified and measured with combinations of HPLC, using [³H]- or [¹⁴C]-radiolabeled substrates, and mass spectrometry (36, 37, 94, 95). Care is necessary because of the instability of these oxidized amine species. The in vitro N-hydroxylation of arylamines and HAAs can be measured colorimetrically by a modification of a Fe³⁺-reduction method using 4,7-diphenyl-1,10-phenanthroline (83, 84, 96). Owing to the inherent instability of aryl N-hydroxylamines and the large numbers of assays required in enzyme kinetic studies (83), this method can be used quite sensitively and routinely.

Many cancer studies on arylamines have been done following the initial epidemiological findings in workers (2, 3), beginning with the induction of urothelial tumors in dogs with 2-NA by Hueper (97).

The carcinogenicity of HAAs has been intensively studied in rodent models. HAAs induce tumors at multiple organs in rats and mice, including liver, lung, colon, small and large intestine, stomach, the hematopoietic system, prostate, mammary gland, Zymbal’s gland, clitoral gland, oral cavity, and urinary bladder (20, 98–100).

ACTIVATION BY P450

Studies of arylamine oxidation go back to the 1940s, with early work on azo dyes by the Millers (101). Several lines of evidence implicated the N-hydroxyl derivatives of the arylamines as being responsible for carcinogenic activity, as well as for the methemoglobinemia induced by some drugs (2, 31). N-Hydroxylation was first demonstrated with the acetamide derivative of 2-AF (102). Subsequently, P450 was demonstrated to be involved in this reaction (103), and further studies extended the work to unsubstituted arylamines (31, 96).

In early work on the multiplicity of P450s, several lines of investigation had suggested the role of Ah locus-linked P450 enzymes (i.e., now recognized as Family 1) in the N-hydroxylation of 2-acetylaminofluorene (AAF) (104, 105). Subsequently,
these P450s were recognized as being involved in the N-hydroxylation of this substrate and several arylamines in various species (32, 33, 106). This is generally the case, with many arylamines preferentially N-hydroxylated by rat P450 1A2 and, to a lesser extent, by P450 1A1 (36). However, 4-ABP is N-hydroxylated by rat P450 proteins in the order 1A2 > 1A1 > 2A6 > 2C11 > 2B1 (36). In the same study, the order for N-hydroxylation of 4,4′-methylene-bis(2-chloroaniline) (MOCA) was 2B1 > 2B2 > 1A2 > 2A6 > 1A1.

The human P450 enzymes involved in the metabolism of arylamines, HAAs, and other chemical carcinogens have long been a subject of interest. Some efforts had been made at analysis with early preparations of human P450s (107). A key development was the purification of the human P450 involved in phenacetin O-deethylation, now recognized as P450 1A2 (108). Analysis of the animal models and subsequent correlations of hepatic expression levels with the N-hydroxylation of 4-ABP (36) led to the view that this enzyme, then known as P450PA, has a major role in the N-hydroxylation of many arylamines and HAAs. Further evidence followed, with the demonstration that the same enzyme is involved in caffeine N3-demethylation (37) and that many HAA activations can be attributed to this enzyme (109). Phenacetin metabolism had been studied in humans in vivo (110), and the characterization of human P450 1A2 (108) led to insight into the inducibility of P450 1A2 in humans. However, phenacetin can no longer be used as a human drug because of concerns about its carcinogenicity, and the demonstration of caffeine N3-demethylation led to the use of caffeine as a noninvasive in vivo probe (37, 111).

The roles of human P450s in the bioactivation of arylamines and HAAs have been considerably documented (24, 37, 109, 112–116). P450s 1A1 and 1A2 have been generally recognized to be the major forms involved in the bioactivation of arylamines and HAAs in human liver and lung microsomes (109, 113, 116). A representative study with HAAs is presented in Table 2. The findings with P450 1A2 have been confirmed in vivo in human studies, at least with PhIP and MeIQx. The P450 1A2–selective inhibitor furafylline blocked most of the in vivo elimination in studies in which the human volunteers consumed burned meat (117). Another P450 Family 1 member, P450 1B1, has been also shown to be an important enzyme involved in the activation of HAAs and has been considered regarding mechanisms of development of human cancers (Table 2) (118, 119). It should be emphasized that some of the P450s (other than Family 1) do have measurable activity with some of the arylamine and HAA substrates. MOCA N-hydroxylation, in contrast with other arylamines, was shown to be preferentially catalyzed by P450 3A4 in human liver (114). Kitada & Kamataki (120) have shown that P450 3A7 can activate some HAAs to mutagens in human fetal liver, where P450 1A2 is not expressed.

Although the early epidemiology linked P450 2D6 with lung cancer incidence (121), subsequent efforts to provide a basis were not fruitful. We have been unable to find any carcinogens that are preferentially activated by P450 2D6, including arylamines, HAAs, and crude cigarette smoke fractions (112, 122).

We have treated P450 1A2 (and other P450s) only in terms of the wild-type (or more correctly, the predominant) allele thus far. The possibility exists that
some individuals will have variants that provide unusual catalytic properties. For instance, we have utilized screens involving the activation of MeIQ to a genotoxic N-hydroxylamine to identify P450 1A2 mutants with high activity in laboratory-generated random libraries (81, 83). Some of these variants have activities (N-hydroxylation) 12-fold higher than the wild type. To our knowledge, none of these has been reported in the population. We have expressed the known allelic variants and found catalytic efficiencies ($k_{cat}/K_m$) for N-hydroxylation of several HAAs within a threefold range, although one P450 1A2 variant failed to incorporate heme and was inactive (84).

Although the discussion is mainly about the activation of arylamines and HAAs, we should also emphasize that P450s are involved in detoxication of the same compounds by other routes (36, 123). This aspect can be important, as shown in the classic Richardson experiment (124) in which enzyme induction by 3-methylcholanthrene lowered the tumorigenicity of an aminoazobenzene compound to rats.

A study with P450 1A2−/− mice indicated that P450 1A2 plays an important role in DNA adduct formation with PhIP and IQ in vivo (125). Differences owing to the absence/presence of P450 1A2 were seen in liver, kidney, and colon but not in mammary glands. However, a neonatal bioassay study with P450 1A2−/− mice suggests that an unknown pathway, unrelated to P450 1A2, appears to be responsible for the carcinogenesis of PhIP (126).

Interspecies differences in metabolism of HAAs by rat and human P450 1A2 were found in the metabolism of MeIQx and PhIP (127, 128). Although rat and human P450 1A2 have 75% amino acid sequence identity (129), relatively high levels of P450 1A2 expression in human liver and catalytic activities for HAA

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### TABLE 2

Activation of HAAs and arylamines by recombinant P450 in an *S. typhimurium*–based genotoxicity system

<table>
<thead>
<tr>
<th>HAA</th>
<th>Concentration, µM&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Activity (umu, units/min/nmol P450)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1A1</td>
</tr>
<tr>
<td>Glu-P-1</td>
<td>1.5</td>
<td>27</td>
</tr>
<tr>
<td>PhIP</td>
<td>220</td>
<td>7</td>
</tr>
<tr>
<td>MeIQx</td>
<td>0.3</td>
<td>16</td>
</tr>
<tr>
<td>MeIQ</td>
<td>0.05</td>
<td>24</td>
</tr>
<tr>
<td>IQ</td>
<td>0.5</td>
<td>10</td>
</tr>
<tr>
<td>Trp-P-1</td>
<td>4</td>
<td>536</td>
</tr>
<tr>
<td>Trp-P-2</td>
<td>4</td>
<td>578</td>
</tr>
<tr>
<td>2-AA</td>
<td>0.1</td>
<td>90</td>
</tr>
<tr>
<td>2-AF</td>
<td>12.5</td>
<td>46</td>
</tr>
</tbody>
</table>

<sup>a</sup>Reference 48.

<sup>b</sup>Lowest concentration used in assay. See original reference for more results (48).
N-hydroxylation compared to the rat P450 1A2 were observed (127, 128). Important differences between human and rat P450s 1A2 were also found in the C8- and N-oxidation of MeIQx (130, 131). These suggest that the interspecies differences in P450 enzyme expression and catalytic activities may be significant and must be carefully considered when assessing human health risk.

The carcinogenicity of IQ, MeIQ, and PhIP was examined in cynomolgous monkeys, with up to seven years of administration (132–134). IQ and PhIP were potent liver carcinogens. IQ and PhIP formed high levels of DNA adducts in a number of organs, particularly the liver, kidney, and heart. However, low mutagenic and carcinogenic activation of MeIQx was observed in this species. The poor activation of MeIQx was explained by the lack of constitutive expression of P450 1A2 and an inability of other P450s to hydroxylate this quinoxaline (133).

Arylamines can also be N-hydroxylated by FMO and peroxidases (32, 33, 135, 136). These reports, along with those demonstrating DNA adduct formation by these routes, suggested that the formation of a common DNA-reactive species could be generated by alternate pathways and these pathways could be considered to contribute to the burden of DNA adducts found in extrahepatic tissues. This appears to be the case because P450 1A2 is not expressed in extrahepatic tissues, and some N-hydroxy HAAs are too unstable to be transported from the liver to distant sites (33–35, 136, 137), although contributions of P450s such as 1A1 and 1B1 may also be an issue.

OTHER BIOCHEMICAL CONSIDERATIONS

The mechanisms of catalysis by P450 are considered elsewhere, including N-hydroxylation (138). N-Oxygenation is considered an inherent part of the repertoire of P450 chemistry available, even in cases where N-dealkylation is preferred (39). A mechanism involving one-electron oxidation of the amine and subsequent homolytic collapse of the intermediated pair is attractive in that there is a basis with accepted mechanisms for other reactions with amines (e.g., N-dealkylation) (138, 139). A problem with this hypothesis is a lack of correlation in the Hammett plots, i.e., limited effect of change in rates owing to the presence of electron-donating groups (39). An alternative is a further one-electron transfer within the intermediate to yield an [Ar-N+/FeO^-] species that recombines (31, 33, 39, 138).

The N-hydroxy products of some HAAs have been observed to further oxidize and produce the nitroso compounds, as judged by autocatalytic NADPH oxidation, reduction cycling, and direct identification of the species (140) (Figure 4). This reaction cycling with human (and also rat and rabbit) P450 1A2 seems to be selective among the HAA substrates and may contribute to toxicity, either through covalent binding to proteins and DNA or possibly oxygen toxicity owing to depletion of NADPH (140). The nitroso derivatives of HAAs react with DNA and protein (26, 43, 140).
Peroxidases generate nitro compounds from HAAs as well as \(N\)-hydroxy products (141, 142). Nitroaromatic hydrocarbons, which are abundant in the environment and can cause cancer (143), undergo nitroreduction to produce \(N\)-hydroxy intermediates in bacteria or in mammalian cells and then follow the same process for mutagenesis as described for amines (77, 112, 136).

RELEVANCE TO DRUG TOXICITY

Today the inclusion of an arylamine moiety in a new drug would trigger a structural alert and, at the least, special attention. However, many older drugs on the market contain the moiety and are in wide use. A number of simple drugs, e.g., phenacetin, are metabolized to nitroso derivatives that have toxicity (144, 145).

SMX causes a variety of unpredictable idiosyncratic drug reactions, including fever, lymphadenopathy, skin rashes, hepatitis, nephritis, and blood dyscrasias in approximately 2%–3% of patients (146). SMX is metabolized not only to stable metabolites, such as the acetamide and glucuronide, but also to a potentially toxic hydroxylamine, which can undergo further oxidation to a nitroso metabolite (SMX-NO) (Figure 5). The \(N\)-hydroxylation of SMX is catalyzed primarily by P450 2C9 in humans (147). Several studies proposed that SMX-NO may be responsible for these idiosyncratic toxicities (26, 148–152).

Dihydralazine has been implicated in sporadic incidence of drug-induced hepatitis (Figure 6). The drug is oxidized by human P450 1A2 and yields autoimmune antibodies (in vivo) that recognize (unmodified) P450 1A2 (153). The relationship of these events to the etiology of the disease remains to be determined.

Dapsone is a drug of choice for treatment of leprosy. Like the simple arylamines, it is known to cause hemolytic problems (154), apparently owing to the \(N\)-hydroxyl and probably nitroso derivatives. Several human P450s have been shown to be capable of dapsone \(N\)-hydroxylation, including P450s 3A4 (155–157), 2E1 (157), and 2C9 (157, 158), with P450 2C9 alleged to be most important at low dapsone concentrations (158).

A general conclusion is that the variability of (human) P450s responsible for \(N\)-hydroxylation of arylamine drugs will be more considerable than for the carcinogenic arylamines and HAAs, based on what is presently known. Thus, a general hypothesis about a role of P450 Family 1 enzymes with these drugs will probably not be tenable.
CONCLUSIONS AND FURTHER QUESTIONS

Human cancer risk associated with HAAs depends on the level of dietary exposure in the population, the biologically effective doses arising from those exposures within relevant target tissues, and the relationship between these effective doses and predicted increased cancer risk (159). Industrial exposure to known carcinogenic
arylamines is probably minimal today, at least in developed nations. Exposure to arylamines does occur via cigarette smoke and hair dyes. Other sources may be an issue in that some arylamine adducts are detected in both smokers and nonsmokers (160–163).

It is difficult but necessary to estimate the risk of HAAs to humans because of the general exposure and the differences of polymorphisms in metabolic enzymes. HAAs have produced tumors in rodent liver, colon, forestomach, Zymbal’s gland, and mammary gland (23) and in nonhuman primate liver. HAA-DNA adducts (PhIP) have been detected in normal human breast and colon (28, 164–166). The exact role of metabolic polymorphisms in the risks of individuals is not yet clear, and consistently epidemiological studies have shown that exposure levels need to be included in evaluations (166, 167). HAAs must be considered major candidates for contributing to human cancer.

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40 KIM GUENGERICH


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CONTENTS

FRONTISPIECE—Minor J. Coon xi

CYTOCHROME P450: NATURE’S MOST VERSATILE BIOLOGICAL CATALYST, Minor J. Coon 1

CYTOCHROME P450 ACTIVATION OF ARYLAMINES AND HETEROCYCLIC AMINES, Donghak Kim and F. Peter Guengerich 27

GLUTATHIONE TRANSFERASES, John D. Hayes, Jack U. Flanagan, and Ian R. Jowsey 51

PLEIOTROPIC EFFECTS OF STATINS, James K. Liao and Ulrich Laufs 89

FAT CELLS: AFFERENT AND EFFERENT MESSAGES DEFINE NEW APPROACHES TO TREAT OBESITY, Max Lafontan 119

FORMATION AND TOXICITY OF ANESTHETIC DEGRADATION PRODUCTS, M.W. Anders 147

THE ROLE OF METABOLIC ACTIVATION IN DRUG-INDUCED HEPATOTOXICITY, B. Kevin Park, Neil R. Kitteringham, James L. Maggs, Munir Pirmohamed, and Dominic P. Williams 177

NATURAL HEALTH PRODUCTS AND DRUG DISPOSITION, Brian C. Foster, J. Thor Arnason, and Colin J. Briggs 203

BIOMARKERS IN PSYCHOTROPIC DRUG DEVELOPMENT: INTEGRATION OF DATA ACROSS MULTIPLE DOMAINS, Peter R. Bieck and William Z. Potter 227

NEONICOTINOID INSECTICIDE TOXICOLOGY: MECHANISMS OF SELECTIVE ACTION, Motohiro Tomizawa and John E. Casida 247

GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE, APOPTOSIS, AND NEURODEGENERATIVE DISEASES, De-Maw Chuang, Christopher Hough, and Vladimir V. Senatorov 269

NON-MICHAELIS-MENTEN KINETICS IN CYTOCHROME P450-CATALYZED REACTIONS, William M. Atkins 291

EPoxide HYDROLASES: MECHANISMS, INHIBITOR DESIGNS, AND BIOLOGICAL ROLES, Christophe Morisseau and Bruce D. Hammock 311
CONTENTS

NITROXYL (HNO): CHEMISTRY, BIOCHEMISTRY, AND PHARMACOLOGY, Jon M. Fukuto, Christopher H. Switzer, Katrina M. Miranda, and David A. Wink 335

TYROSINE KINASE INHIBITORS AND THE DAWN OF MOLECULAR CANCER THERAPEUTICS, Raoul Tibes, Jonathan Trent, and Razelle Kurzrock 357

ACTIONS OF ADENOSINE AT ITS RECEPTORS IN THE CNS: INSIGHTS FROM KNOCKOUTS AND DRUGS, Bertil B. Fredholm, Jiang-Fan Chen, Susan A. Masino, and Jean-Marie Vaugeois 385

REGULATION AND INHIBITION OF ARACHIDONIC ACID (OMEGA)-HYDROXYLASES AND 20-HETE FORMATION, Deanna L. Kroetz and Fengyun Xu 413


PROTEASOME INHIBITION IN MULTIPLE MYELOMA: THERAPEUTIC IMPLICATION, Dharminder Chauhan, Teru Hideshima, and Kenneth C. Anderson 465

CLINICAL AND TOXICOLOGICAL RELEVANCE OF CYP2C9: DRUG-DRUG INTERACTIONS AND PHARMACOGENETICS, Allan E. Rettie and Jeffrey P. Jones 477

CLINICAL DEVELOPMENT OF HISTONE DEACETYLASE INHIBITORS, Daryl C. Drummond, Charles O. Noble, Dmitri B. Kirpotin, Zexiong Guo, Gary K. Scott, and Christopher C. Benz 495

THE MAGIC BULLETS AND TUBERCULOSIS DRUG TARGETS, Ying Zhang 529

MOLECULAR MECHANISMS OF RESISTANCE IN ANTIMALARIAL CHEMOTHERAPY: THE UNMET CHALLENGE, Ravit Arav-Boger and Theresa A. Shapiro 565

SIGNALLING NETWORKS IN LIVING CELLS, Michael A. White and Richard G.W. Anderson 587

HEPATIC FIBROSIS: MOLECULAR MECHANISMS AND DRUG TARGETS, Sophie Lotersztajn, Boris Julien, Fatima Teixeira-Clerc, Pascale Grenard, and Ariane Mallat 605

ABERRANT DNA METHYLATION AS A CANCER-INDUCING MECHANISM, Manel Esteller 629
