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Highlights of the Eighth International Conference on Carcinogenic/Mutagenic *N*-Substituted Aryl Compounds

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Abstract

Research in the 20th century initially identified arylamines as causative factors in occupational carcinogenesis, especially bladder cancer, and subsequently identified arylamines as a major class of mutagens/carcinogens in the environment and diet that are potential risk factors in a variety of human cancers. Current research focuses on understanding of mechanisms of arylamine carcinogenesis, such as the role of metabolic processing, DNA adduct formation, and mutagenesis, and learning more about the molecular alterations in carcinomas induced by these compounds. Furthermore, research to identify human exposures, including developing more sensitive methods for analyzing environmental samples and identifying suitable biomarkers are important aspects of contemporary investigations. In addition, better evaluation of the risk of these compounds in human cancer especially with regard to the impact of genetic polymorphisms is a major focus of research in this field. Although current population studies have sometimes been described as equivocal, improved tools for epidemiology, refined human biomonitoring methods and collaborative endeavors to study multiple population groups now provide a better means to ultimately define the role of arylamines in human carcinogenesis. The purpose of the Eighth International Conference on Carcinogenic/Mutagenic *N*-Substituted Aryl Compounds, held in Washington, DC, 12–14 November 2001, was to explore the current scope of studies on arylamine carcinogenesis among scientists in basic research and epidemiology and to discuss future research priorities. With the intent of providing a view to the current field of research on aromatic amines, this review presents a synopsis of the Proceedings of the Eighth International Conference and highlights the manuscripts contained in this special issue of *Mutation Research*.
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1. Aromatic amine exposure and carcinogenesis

Over 100 years ago, urinary bladder cancer in men was associated with occupational exposure to aromatic amines in the dye industry [1,2]. By the early to mid 1900s, it became clear that certain arylamines used

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in the dye industry, such as 2-naphthylamine, benzidine, and 4-aminobiphenyl were causative agents in human bladder cancer. Although the era of high dose exposure has largely passed, aromatic amines are still found in the chemical, dye, and rubber industries, as well as in hair dyes, fungicides, cigarette smoke, diet and automobile exhaust. In addition, present-day levels of aromatic amine exposure continue to be linked to human bladder cancer and other cancers [1,3–8]. As discussed at the conference, 4-aminobiphenyl in cigarette smoke and aromatic amines in hair dyes have been associated with human bladder cancer [3,4]. Current studies are directed to assessing the human cancer risk after relatively low-level exposures, learning more about potential sources of human exposure, the target organs for arylamine carcinogenesis in humans, and methods to minimize arylamine exposures [3,9]. Although arylamines were first linked to bladder cancer, carcinogenicity studies of "classical" aromatic amines as well as studies of the more newly identified heterocyclic amine food mutagens (discussed in some detail below) indicate potentially multiple target sites for arylamine carcinogenesis in humans [1,2]. For example, 2,6-dimethylaniline, a major metabolite of the drug lidocaine, is a nasal carcinogen in rats [7], and 4-aminobiphenyl is a mammary gland carcinogen in rats ([2], Snyderwine, unpublished observations). Malachite green, widely used as an antifungal in the fish industry, is under investigation for hepatocarcinogenicity [10].

It has been known for many years that metabolic processing is critical for arylamine carcinogenesis. Featured at the conference were studies on the family of *N*-acetyltransferases, polymorphic enzymes that have a primary role in the activation and/or deactivation of the aromatic amines [11,12]. Research on the *N*-acetyltransferases has been central to studies of aromatic amine carcinogenesis and risk assessment for many years, especially with regard to urinary bladder carcinogenicity, and more recently in studies of heterocyclic amine carcinogenesis at several target sites. The high frequency of acetylation polymorphisms in the human population has stimulated the analysis of these enzymes as modifiers of human cancer susceptibility. *N*-Acetylation phenotype, specifically slow *N*-acetyltransferase 2 phenotype, was shown many years ago to modify human bladder cancer risk, and the association was strongest when exposure to certain aromatic amines was documented [11,12]. The *N*-acetyltransferases, of which there are now known to be over 50 allelic variants in humans, are currently prominent enzymes in studies of gene–environment interactions [11,12]. Current and future studies are concerned with the molecular and biochemical characterization of the large family of *N*-acetyltransferase allozymes. Studies directed to better understanding the relationship between acetylator genotype and phenotype and their contribution to human cancer risk from aromatic amine exposure are at the forefront of research in this field [3,5,11].

2. Mutagenesis and molecular alterations

Mutagenesis studies have been of long-standing importance to the field of aromatic amine research. DNA adduct formation and the induction of mutations are now recognized to be critical for the carcinogenicity of these compounds [13]. It is also notable that the cooked meat-derived heterocyclic amines, which have spurred research in this field especially over the last 10–15 years, were initially identified by their potent mutagenicity in the Ames Salmonella assay [14]. Study of the mutation spectra associated with specific arylamine DNA adducts has been and still is an active area of research [7,10,15–17]. Research is currently focused on understanding the detailed mechanisms of arylamine mutagenesis and the role of specific mutations in susceptibility to carcinogenesis [10,15,16]. The structural requirements for mutagenic potency are being examined with the heterocyclic amines [17,18]. Computational modeling and experimental methods are being used to study the reaction mechanism involved in arylamine *N*-oxidation to the arylnitrenium ion, the putative reactive intermediate associated with DNA adduct formation and mutagenesis [18].

The molecular and genetic toxicology of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) is another currently active area of investigation [19–22]. Considerable emphasis is being placed on PhIP since it is the major known carcinogenic heterocyclic amine in the human diet [23]. Genetic toxicity was shown to be dependent on metabolic activation, especially by the CYP1 family of cytochrome P450s and specific phase II esterification enzymes including *N*-acetyltransferases and sulfotransferases, as well

as affected by DNA repair capacity. In addition to providing valuable insight into mechanisms of carcinogenesis, these experimental studies continue to provide rationale for molecular epidemiology studies investigating metabolic processing, DNA adduct formation, mutation spectra and cancer risk. In vitro genetic toxicology studies have also recently indicated that besides mutagenicity, PhIP had effects on cell cycle and cell growth, including an estrogenic activity, that are potentially relevant to novel mechanisms of carcinogenesis [22]. In addition, the effect of PhIP on cell signaling and modulation of hormone responses might be pertinent to its mechanisms of carcinogenesis in specific target organs [24]. Further studies are required to investigate whether the carcinogenic mechanism of action for these compounds involves interaction with hormone receptors and whether this interaction might facilitate carcinogenesis in androgen/estrogen sensitive organs. It has been claimed that PhIP and many of the estrogen receptor agonists have relatively similar structures [22]. This needs to be clarified and remains to be studied further by sophisticated computational-chemical methods.

Current research is also focused on elucidating the mutations and genomic alterations in target tissues and carcinomas, especially mammary gland carcinomas, induced by PhIP and other heterocyclic amines. There is evidence that PhIP–DNA adducts induce characteristic mutational changes [16,25,26]. In mammary gland carcinomas, PhIP is also associated with an increased rate of spontaneous point mutations, characteristic of single nucleotide instability [27,28]. Additional studies in this area are expected to provide further insight into mechanism of mammary gland carcinogenesis. Other investigators have reported that PhIP-induced rat mammary gland carcinomas harbor a distinctive cytogenetic signature as detected by comparative genomic hybridization [29]. Further study of the chromosomal regions found consistently lost in PhIP-induced carcinomas should help to identify the genes important for mammary gland cancer development by this agent. Current evidence concerning PhIP-induced mutation spectrum in several target organs supports the feasibility of using molecular information and mutation spectra data for risk estimation [25]. In future, it may be possible to confirm the role of PhIP and other aromatic amines in human cancers from the characteristic genomic alterations induced.

3. Experimental models and modulation of carcinogenesis

Experimental models are clearly allowing a better understanding of the mechanisms of carcinogenic targeting of arylamines. Many experimental models are being used to investigate arylamine carcinogenesis and highlighted in this conference were approaches for studying colon, mammary gland, and prostate carcinogenesis in rats and transgenic mice [30–37]. These three cancer sites show relatively high incidence in the Western world and are partly associated with dietary factors, such as cooked meat consumption and possibly heterocyclic amines as discussed further below. In addition to the currently recognized heterocyclic and other aromatic amines, a group of investigators have recently identified aminophenylnorharman, a novel aromatic amine, and are currently investigating the in vivo formation, mutagenicity, and carcinogenicity of this compound [9].

From experimental studies, the critical factors involved in arylamine carcinogenesis can be dissected in ways not general feasible in human studies. To date, multiple determinants of carcinogenesis including dose of the arylamine, production of reactive metabolites, DNA adduct formation, promotion, tissue growth and differentiation, and genetic susceptibility have been shown to be important for arylamine carcinogenesis. It is notable that the mechanisms of carcinogenesis are also likely to be somewhat different between target organs [34]. Current experimental carcinogenesis studies are increasingly at the molecular level and involve the analysis of specific genes and cell signaling pathways. Evidence now suggests a role for alterations, such as mutations and loss of heterozygosity, in the beta-catenin and *APC* genes in colorectal cancers induced by PhIP and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) [30–32]. The susceptibility of different strains of rats to colorectal cancer induced by PhIP is also being examined and is yielding novel information about the genetic traits affecting the development of this disease [35].

Studies concerning the modulation of arylamine carcinogenesis by potential chemopreventive agents are also at the forefront of research, and indeed they have been an active area of research with the heterocyclic amines over the last 5–10 years. Research

is currently addressing the impact of chemopreventive agents including dietary components, such as phytochemicals, on various molecular end points, such as mutations in critical genes and alterations in gene expression. For example, the chemopreventive effect of tea polyphenols on colorectal cancers induced by PhIP in transgenic mice may in part involve modulation of the beta-catenin pathway [30]. These current approaches are providing new information about the specific molecular alterations involved in arylamine carcinogenesis and may potentially facilitate chemopreventive strategies taylored to a specific etiological agents and a particular cancer site.

In addition to colorectal cancer, experimental approaches for studying mammary gland carcinogenesis, have been prominent in the field of aromatic amine research [36,38,39]. Since the earliest reports of its mammary gland carcinogenicity in rats [40] and its excretion into breast milk [41], PhIP has been implicated as a human mammary gland carcinogen [40–42]. Detailed studies using animal models are providing insight into mechanisms of mammary gland carcinogenesis of PhIP including the pathways of metabolism, DNA adduct formation, mutations, effects on circulating hormones and cell signaling [36]. The many experimental studies being carried out with heterocyclic amines and other aromatic amines including the studies reported in this special issue are focused on dissecting the contribution of these many factors in target organ specificity.

4. Biomonitoring, DNA adducts and metabolism in humans

Recent advances in methodology have facilitated the identification of arylamines and arylamine metabolites in bodily fluids, such as urine and breast milk of people [43–47]. Employing techniques, such as LC/MS/MS, analysis of urine from individuals ingesting fried or charbroiled meats rich in heterocyclic amines has detected inter-individual differences in metabolic processing [43–46]. In addition to the *N*-acetyltransferases discussed above, metabolic processing by human cytochromes P450, sulfotransferases, and glutathione *S*-transferases and the glucuronyltransferase families of enzymes are understudy. All these families of enzymes show polymorphisms and inter-individual variation and are at the forefront of studies relating metabolic processing and specific metabolic phenotypes to human cancer risk [11,46,48,49]. Studies comparing metabolism of heterocyclic amines by rat and human CYP1A2, a major isoform associated with the proximate reactive *N*-hydroxylamine metabolite, have yielded valuable information regarding species differences by showing a significantly higher catalytic activity of the human isoform [50]. The glucuronide conjugates of PhIP and its proximate reactive metabolite, *N*-hydroxy-PhIP, have been recently quantitated in people eating cooked meats and studies to date suggest that urinary *N*-hydroxy-N^2-glucuronide may serve as a biomarker of the degree of bioactivation and detoxification of this reactive metabolite [45,46]. Ultimately, metabolic profiling and knowledge of the impact of specific polymorphisms in genes involved in arylamine metabolism are expected to help link arylamine exposure with specific human cancers and identify high-risk individuals.

Aromatic amine DNA adduct formation in target tissues is an ideal biomarker for confirming exposure, metabolic activation, and possible genotoxicity [13], however there have been challenges with measuring adduct levels in people exposed to environmentally-relevant levels of carcinogen. Several groups of investigators are pursuing the measurement and quantitation of arylamine DNA adducts in human tissues and exfoliated cells by techniques, such as ^{32}P-postlabeling and accelerator mass spectrometry [51–54]. For the purpose of human biomonitoring, methodology is being developed and is undergoing further refinement to quantify DNA and protein adducts in human tissues [55]. Factors that affect human heterocyclic amine intake, e.g. types of meat, cooking time, as well as the influence of other dietary components on metabolic processing and excretion are being actively investigated [43]. To date, studies suggest that specific dietary components may affect the rate of metabolism and excretion of carcinogenic heterocyclic amines in humans. Future research in this area is likely to provide insight into methods to minimize heterocyclic amine formation, as well as methods to modulate in vivo carcinogenesis with dietary components that may facilitate detoxification.

5. Human risk from exposure to dietary heterocyclic amines

It should be apparent from the preceding paragraphs, that dietary heterocyclic amines are the major class of aromatic amines currently being studied in this field. Although, the carcinogenicity of heterocyclic amines has been clearly established in animal studies, there have been challenges in assessing human risk to heterocyclic amine exposure [56]. These challenges as well as approaches for future studies were discussed in depth at the conference in a session that focused specifically on human risk from heterocyclic amines. Some of these hurdles have included the development of methodology and questionnaires to assess human exposure, and the designing of studies to evaluate the contribution of specific genetic polymorphisms in metabolic processing. To date, studies presented in this session and reported in this special issue discuss the evidence for an association of heterocyclic amines with cancers of the colorectum [57,58], breast [59], prostate [60,61], lung [62], and pancreas [63]. These associations were further strengthened in studies that also considered metabolic phenotype. High exposure to heterocyclic amines, rapid CYP2A6 phenotype, low activity hGSTA1 and low activity SULT1A1 alleles all appear as risk factors [48]. In addition, an interaction between well-done red meat, cigarette smoking, *N*-acetyltransferase and CYP1A2 rapid phenotypes were suggested [58] and warrants further examination in larger population studies. In the future, further studies addressing important methodological challenges in epidemiology especially with regard to gene–environment interactions are likely to be critical for improving the ability to draw valid conclusions concerning the risk of heterocyclic amine exposure. In order to obtain valid results for such interactions, very large well-designed studies with adequate power (number of cases in the hundreds or possibly thousands, depending on genotype prevalence) are needed. In this special issue, several papers discuss the problems encountered to date in human studies and provide more precise analytical approaches to epidemiologic assessment [56]. Biomonitoring in association with improved questionnaires, plus collaborative studies across different population groups [61,64,65], and the inclusion of international studies are tenable approaches in future studies to assess risk. Indeed, the question of the role of dietary heterocyclic amines in human cancer risk may be best answered through large cohort studies that are followed for a number of years.

6. Conclusions

In this volume, selected papers from the Eighth International Conference provide an up-to-date perspective on the research currently ongoing in the field of aromatic amines. As evidenced by these papers, there has been a wide expansion of research and considerable progress made in this field in the last 3–4 years. Research efforts in this field are now directed to further understand carcinogenic and mutagenic mechanisms. These include genomic alterations associated with arylamine exposure, better evaluating the sources and levels of human exposures, identifying specific biomarkers of human risk and critical pathways of metabolic processing in humans, and elucidating factors that modulate the risk of arylamine carcinogenicity. The research being carried out today stands on the shoulders of seminal aromatic research describing, for example, the identification of 2-naphthylamine, 4-aminobiphenyl, and benzidine as etiological agents in human bladder cancer, and the early studies that both established the overall importance of metabolic activation and further defined the major routes of metabolic activation required for arylamine carcinogenesis. Over the past 25 years, there has been considerable expansion specifically in the area of the heterocyclic amines because of their presence in cooked meat and their potential relevance to cancers linked to diet. The challenge for this field in the 21st century is to ascertain the impact of current levels of arylamine exposure on the incidence of human cancers, better understand the processes involved in arylamine carcinogenesis, and subsequently determine the approaches to minimize human cancer risk from these etiological agents.

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