Journal of Cellular Biochemistry, Supplement 17F:138–148 (1993)

DNA Adducts in Model Systems and Humans

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Abstract The etiology of chemically induced cancer is thought to involve the covalent binding of carcinogens to DNA (adducts) leading to mutations in oncogenes or tumor suppressor genes, and ultimately to tumors. Thus, the DNA-carcinogen adduct has been used as a measurable biochemical endpoint in laboratory studies designed to assess carcinogen exposure, carcinogen metabolism, mutagenesis, and tumorigenesis. Unfortunately, the significance of adducts in the etiology of human cancer is still unclear. This is partially due to the difficulty detecting adducts at carcinogen exposures relevant to humans, which are often orders of magnitude lower than animal model exposures. The relationship between adducts and higher biological effects is also not known at low doses. We have been assessing the DNA damage caused by exposure to heterocyclic amine carcinogens in the diet. Using the technique of ^{32}P-postlabeling in combination with accelerator mass spectrometry, we have determined that DNA adduction in rodents decreases linearly with decreasing dose from the high doses used in typical cancer bioassays to the low doses relevant to human exposures. For a given tissue, adduct levels are correlated with dose, but the level of DNA modification by carcinogens is tissue-specific and does not completely correlate with tumor site. This lack of correlation may be due to differences in adduct formation and repair rates among tissues. Comparison of carcinogen metabolism routes between rodents and humans also indicates that species differences could influence the amount and type of damage resulting from exposure to these carcinogens. The use of model systems to study dosimetry, species differences in adduction, and role of adducts in mutation will ultimately lead to a better understanding of the significance of adducts in human disease. This should eventually allow the use of adducts as biomarkers for estimating carcinogen exposure and individual susceptibility.

Key words: Adduct dosimetry, MeIQx, PhIP, accelerator mass spectrometry, ^{32}P-postlabeling, DNA adducts

Adduction is the covalent bonding of xenobiotics or indigenous chemicals with macromolecules. Adduction most often occurs after chemicals metabolize to reactive electrophilic or nucleophilic intermediates (bioactivation). These intermediates then covalently bind to their respective nucleophilic or electrophilic centers in macromolecules such as DNA or protein. DNA adduction is thought to be the initiating event in mutagenesis and chemical carcinogenesis, and is of great interest as an indicator of exposure, individual susceptibility, and biological effect [1]. DNA adducts are very useful endpoints in mechanistic studies designed to understand the modulation events leading to mutagenesis and tumorigenesis. This results from the fact that adduct levels are the integrated result of carcinogen absorbtion, carcinogen metabolism, and adduct repair. DNA adduction

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may also be a target for intervention modalities to reduce individual cancer risk.

We have used a number of models, including whole animals, tissue fractions, and bacterial assays, to study the mechanisms leading to DNA adduct formation, species differences in these mechanisms, and the dosimetry of DNA adduction. In particular, we have examined a group of dietary heterocyclic amine carcinogens that may be causative factors in the incidence of human colon cancer [2]. We have principally used the techniques of accelerator mass spectrometry (AMS) and ^{32}P-postlabeling. AMS is a low-energy nuclear physics technique for measuring isotopes, making it ideal for isotope tracer studies [3]; it isolates and counts specific nuclei particle by particle as opposed to measuring atomic decay. Depending on the isotope, AMS gives a 10^3- to 10^6-fold improvement in isotope detection efficiency relative to decay counting, allowing us to quantitatively measure DNA adduct levels on the order of one adduct per 10^{11}–10^{12} nucleotides (1 adduct/100–1000 cells) [3,4]. This sensitivity has made possible studies on DNA adduction at doses of chemical carcinogens close to actual human exposures from environmental sources. ^{32}P-postlabeling is an assay for DNA adduct detection that uses thin-layer chromatographic (TLC) separation of specific adducted nucleotides after the DNA is digested to deoxyribonucleotides [5]. In practice, the method has a sensitivity of 1 adduct per 10^8–10^{10} nucleotides, corresponding to approximately 0.1 to 10 DNA adducts per cell [5]. The assay's principal use is in analyzing a large complement of structurally diverse adducts without prior knowledge of the adduct structure or exposure. It is very useful in studies to assess mechanisms and levels of DNA binding and potential human exposures.

DIETARY HETEROCYCLIC AMINE CARCINOGENS

A great deal of concern has been expressed recently that cooking meat produces genotoxic substances which may contribute to the incidence of GI tract or other human cancers. Of all the substances known to be produced during cooking, the most important may be the heterocyclic amines. These compounds are considered significant because they are produced at temperatures needed to grill, fry, and broil red meats, poultry, and fish [2,6]. The heterocyclic amines formed in meat through cooking have been collectively referred to as the amino-imidazoazaarenes (AIA); they consist of several quinolines, quinoxalines, and a phenylpyridine (Fig. 1). Fourteen have been identified to date [2]. All are formed from the condensation of amino acids with creatin(in)e during pyrolysis [7]. These heterocyclic amines may also occur in cigarette smoke, although the precursors are currently undefined [8,9]. Importantly, all heterocyclic amines are mutagenic in bacterial assays [10]. Nine have been tested and shown to be carcinogenic in rodents [11]; one, 2-amino-3-methylimidazo(4,5-*f*)quinoline (IQ), has been shown to be a potent inducer of hepatocellular carcinomas in non-human primates [12].

HETEROCYCLIC AMINE-DNA ADDUCT DOSIMETRY

As with most environmental carcinogens, the cancer risk posed to humans by heterocyclic amines in the diet is difficult to ascertain accurately. Studies in laboratory animals have been traditionally carried out at doses well in excess of actual human exposure levels. Human exposure to these environmental agents occur at the part-per-billion to part-per-trillion level. The rodent and non-human primate bioassays have been carried out at doses at least 10,000-fold greater [11,12]. Metabolism studies, DNA binding studies, and tissue distribution studies have all been done at doses in excess of 1,000-fold greater than those anticipated for human exposure. Thus, it is important to understand the specific effects of the chemicals at human exposure levels so that realistic risk estimates become possible.

To assess the relationship between the binding of heterocyclic amine carcinogens to DNA at the high doses used in traditional cancer bioassays and the low doses relevant for human dietary exposure, we have used ^{14}C-labeled AIA molecules and the isotope detection sensitivity offered by AMS. Administration of ^{14}C-MeIQx [2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline] to rodents at doses ranging from 5 mg/kg to 5 pg/kg (ig), followed 24 hours later by isolation of hepatic DNA, showed that adducts decrease linearly with decreasing dose [4]. Since exposure

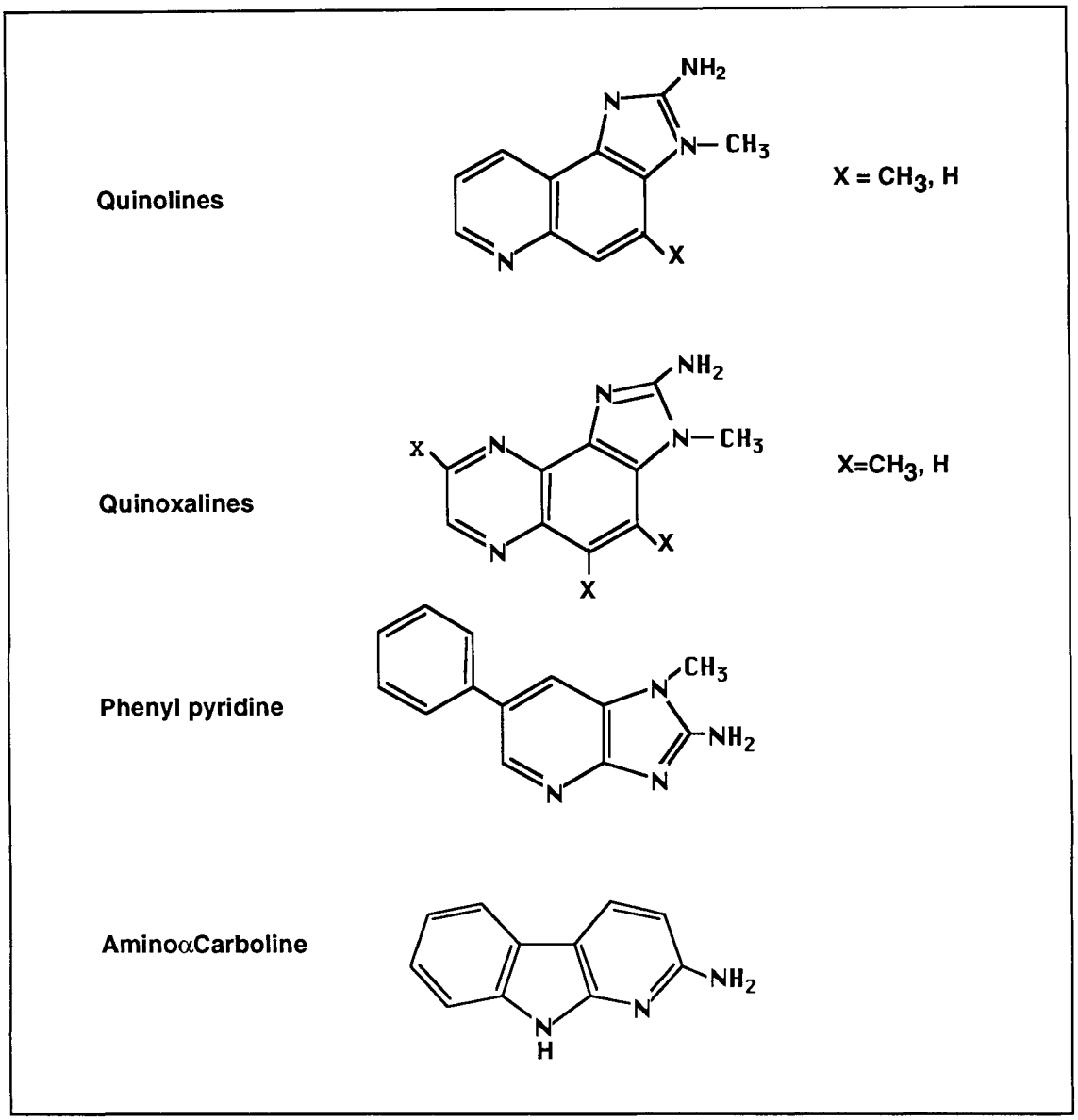

Fig. 1. Classes of amino-imidazoazaarene heterocyclic amines found in the western-type diet. Fourteen specific compounds, varying in number and location of the methyl groups, have been identified to date.

to these heterocyclic amines is chronic rather than acute, we have also investigated the kinetics of chronic exposure to MeIQx (Fig. 2). ^{14}C-labeled MeIQx was administered to rodents daily in the diet at a dose of 100 ng/kg/day to mimic the human exposure route and concentration. Adduct levels were detectable 24 hours after ingestion and continued to increase in the liver and kidney; by 42 days, adduct levels had achieved a steady-state level. Tissue concentrations of MeIQx reached steady-state in 7 days. Adduct levels at steady-state in the liver were 16 ± 1.4 adducts/10^{11} nucleotides for this dose. Sub-chronic (7-day exposure) dose-response data, again covering the dose range between relevant human exposure and the exposure used in cancer bioassays, suggests a linear dose-response for adducts and administered dose

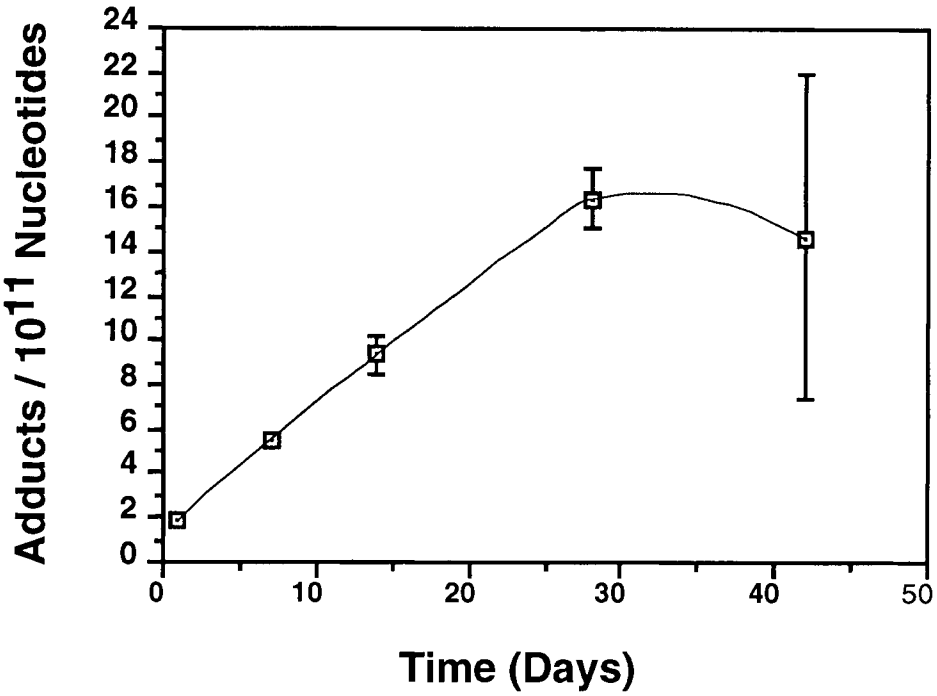

Fig. 2. Kinetics of MeIQx-DNA adduct. Animals were given ^{14}C-MeIQx (2-amino-3,8-dimethylimidazo(4,5-*f*)-quinoxaline) in the diet (100 ng/kg/day). DNA adduction was determined using accelerator mass spectrometry.

(Fig. 3). The biologically available doses present in the tissues also decreased linearly with decreasing dose (biologically available dose). These data suggest that DNA adducts are present at human exposure levels and are indicative of exposure dose for this carcinogen. Longer-term chronic dose-response studies are now in progress with this heterocyclic amine.

Analysis of DNA adducts among tissues of rodents given the heterocyclic amines 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP), MeIQx, and IQ by ^{32}P-postlabeling show that the slope of the dose-response is dependent on the tissue type. In studies conducted in our laboratory, the pancreas yields the greatest level of adducts with PhIP and also the greatest slope of the dose-response curve (Fig. 4). Although preliminary in nature, these data are important because high levels of adducts are found in the pancreas and thymus, not in the liver. In direct contrast, MeIQx and IQ result in high levels of adducts in the liver relative to other tissues [13,14]. It is also interesting that PhIP does not cause liver tumors while MeIQx does [15,16]. Although PhIP generates high levels of adducts in the pancreas, it does not cause pancreatic tumors [15]. Thus, DNA adduct levels of heterocyclic amines correlate with exposure but are not highly correlated with tissue-specific tumor development. Other factors, such as DNA repair, in the tissues will likely influence these relationships and needs to be investigated.

MECHANISTIC STUDIES

Metabolism of DNA-Binding Intermediates Using Tissue Fractions and Bacteria

A number of *in vitro* systems have been used to identify key metabolic steps in the activation of these carcinogens to DNA-adducting intermediates. Metabolism of heterocyclic amines is required for DNA adduction. This process has been shown to involve *N*-hydroxylation by the P-450IA family of cytochrome P-450 [17,18]. Studies using reconstituted P-450 isozymes purified from rodents and humans have demonstrated that P-450IA2 is the principal isozyme carrying out the *N*-hydroxylation of these compounds, followed by P-450IA1, and that the

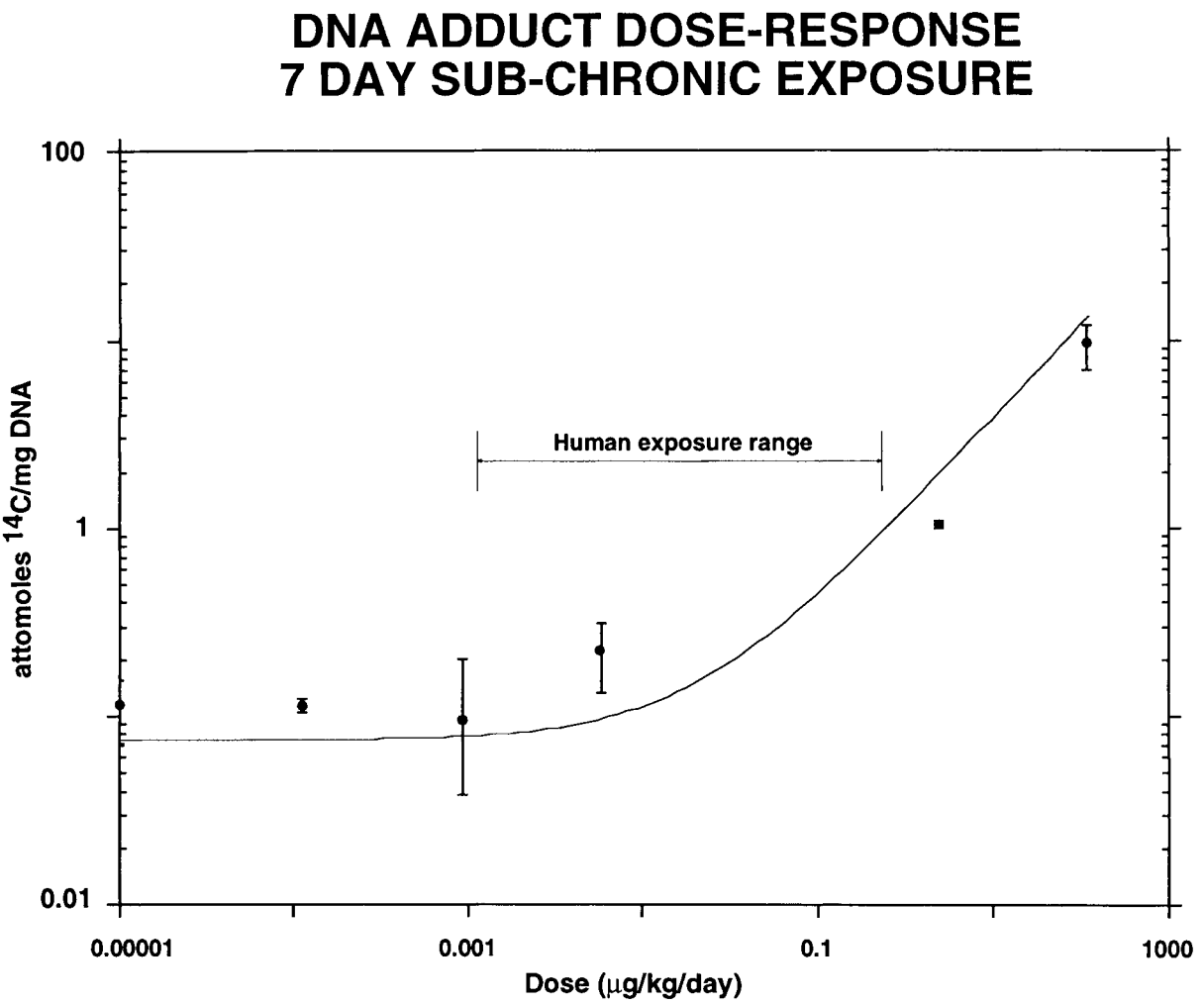

Fig. 3. Sub-chronic (7-day) rat liver DNA adduct dosimetry found with MeIQx. Rats were administered varying doses of ^{14}C-labeled MeIQx (2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline) for 7 days in the diet. DNA from the liver was analyzed for adduct levels using AMS. Response was linear from doses used in rodent cancer assays down to doses relevant for human exposure ($y = 5.2278^{-14} + 2.7449^{-10}x$, $R = 0.99$). Human daily exposure range is shown for consumption of 100–200 g of ground beef/day.

2-*N*-hydroxylamine is the principle mutagenic product formed by these enzymes in animal tissue fractions [19].

Further metabolism involves esterification of the *N*-hydroxylated amine to biologically active acetates, sulfates, and other possible conjugates [17]. These conjugates appear necessary for adduction *in vivo*. Further, these metabolic pathways are compound-specific [17,20,21]. DNA binding directly with the *N*-hydroxyl amines can be shown to occur, but with relatively low affinity. Rather, studies with the quinoline and quinoxaline heterocyclic amines have shown that the compounds are mutagenic to *Salmonella* strains TA98 and TA98NR (nitroreductase deficient); but not to TA98/1,8DNP$_6$ (acetyltransferase deficient), suggesting a requirement for *N*:*O*-acetyltransferase (Fig. 5). DNA adduct analysis by ^{32}P-postlabeling of these bacterial strains show that there is no difference in adduct levels between the nitroreductase-deficient and the parent strains, but adduct levels are significantly lower in the *N*:*O*-acetyltransferase-deficient *Salmonella*

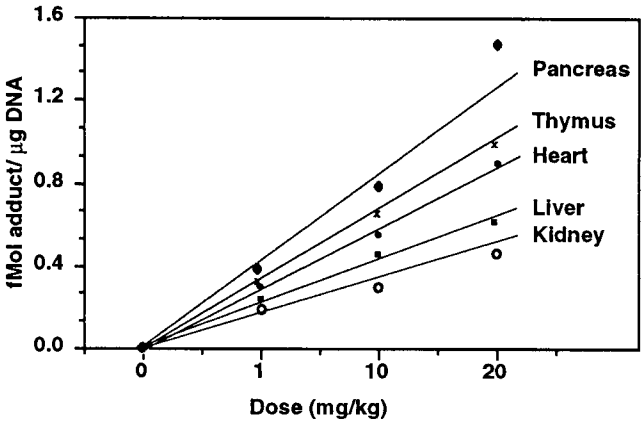

Fig. 4. PhIP [2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine]-DNA adduct dose-response among tissues of the C57Bl/6 male mouse 24 hours after exposure. Adduct levels were greatest in the pancreas, followed by the thymus and heart. Adducts were measured by ^{32}P-postlabeling [22].

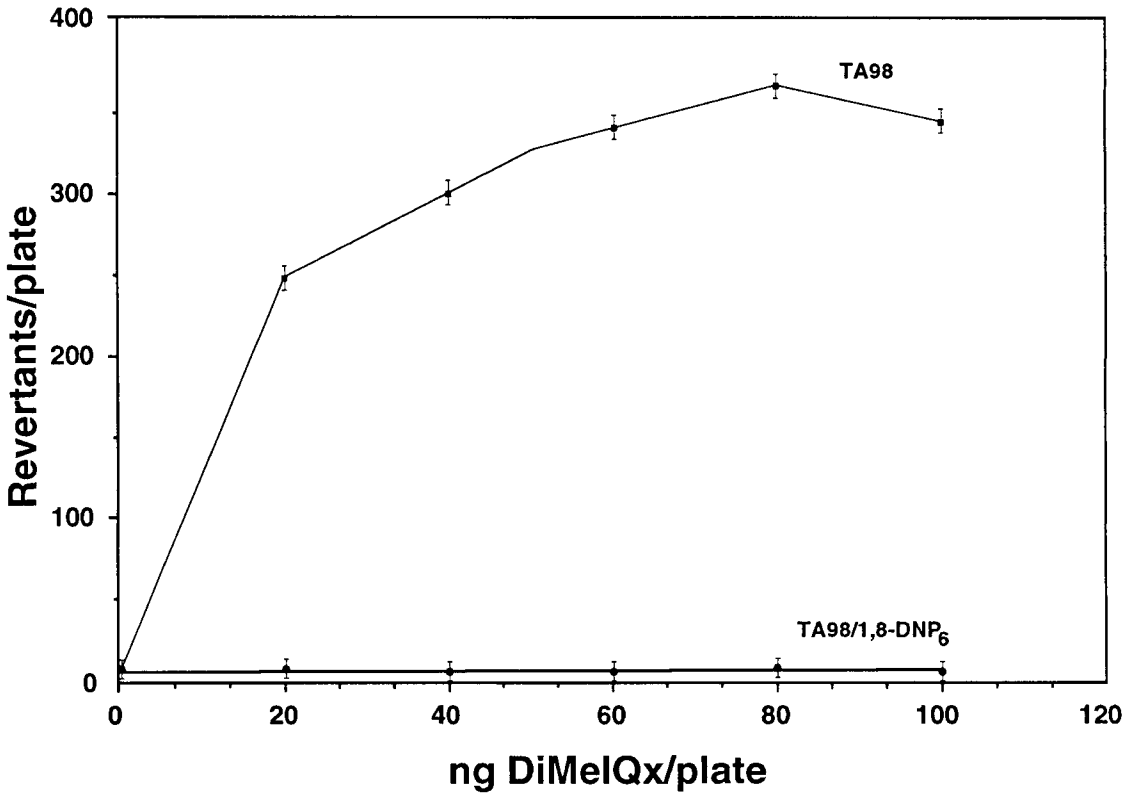

Fig. 5. Effect of *N*:*O*-acetylation capacity on the mutagenicity of 2-amino-3,4,8-trimethylimidazo(4,5-*f*)-quinoxaline (4,8-DiMelQx) in the Ames *Salmonella* assay. *Salmonella* TA98 is *N*:*O*-acetyltransferase competent, while the mutant strain TA98/1,8DNP is *N*:*O*-acetyltransferase deficient [30]. The Ames *Salmonella* assay was carried out as described [31].

strain, suggesting a requirement for *N*:*O*-acetylation [22]. Similar results were found with IQ [23,24]. PhIP, however, is equally mutagenic in all three of these *Salmonella* strains. DNA adduction can be seen in sulfate as well as acetate-supplemented cytosolic enzyme-mediated reactions, suggesting that PhIP does not absolutely require *N*:*O*-acetylation for mutagenicity and DNA adduct formation [17,21].

Besides specific routes of metabolism, studies of DNA adduction have demonstrated that metabolic capacity influences adduct levels. This can be demonstrated by the effects of several agents known to increase the levels of P-450 isozymes (P-450 inducers) on DNA adduct generation in rodents exposed to MeIQx [22]. Metabolic capacity was examined because earlier studies from our laboratory showed that polychlorinated biphenyl (PCB)-induced rodent liver S9 and human liver S9 were more similar in their ability to activate heterocyclic amines to mutagens in the Ames *Salmonella* assay than they were to S9 from non-induced animals. In these studies, MeIQx was administered following induction with phenobarbital (PB), Arochlor 1254, β-naphthoflavone (BNF), or corn oil. DNA was isolated from the livers 24 hours after exposure to MeIQx and analyzed by ^{32}P-postlabeling analysis. Induction by Aroclor and BNF produced DNA with significantly more adducts than either the corn oil- or PB-treated animals. Both corn oil- and PB-treated animals were similar. These data suggest that P-450IA type isozymes are involved in the activation of MeIQx *in vivo* and that the metabolic capacity of the animal does affect the rate of DNA adduction.

Biointoxification of DNA-Damaging Intermediates in Human Tissue Fractions

The role of these pathways in activating heterocyclic amines by humans has been under investigation. Studies using human microsomal fractions show that *N*-hydroxylation is catalyzed by cytochrome P-450IA [25,26]. *In vitro* studies in our laboratory use systems containing calf thymus DNA to trap unstable electrophilic intermediates. Human cytosolic fractions have demonstrated that *N*-hydroxy-PhIP is *N*:*O*-acetylated and *N*:*O*-sulfated but that *N*:*O*-acetylation of PhIP is more active than *N*:*O*-sulfation (Fig. 6). In rodent cytosols, sulfation was quantitatively more important than acetylation [21]. Examination of tissue specificity in these metabolic reactions demonstrated that human liver cytosol is the most active for both acetylation and sulfation of the *N*-hydroxylated intermediate of PhIP (Fig. 6). Such species and tissue differences may, depending on the stability of the intermediate and the rates of activation, result in differences in potency of the heterocyclic amines in DNA adduction and, ultimately, cancer risk. In addition, the fact that metabolism is most active in liver fractions while adduct levels are low and tumors are infrequent suggests that active metabolites may circulate throughout the organism.

Chemical Characterization of the Heterocyclic Amine DNA Adducts

The chemical structures of several heterocyclic amine DNA adducts have recently been determined through use of *in vitro* incubations of DNA or deoxynucleotides with acetylated *N*-hydroxyl-AIA intermediates. ^{32}P-postlabeling of DNA from animal and bacterial models indicates that between 3 to 4 principal DNA adducts are formed by most AIA heterocyclic amines with DNA *in vivo* [13,14,21–23]. To date, an *N*-(deoxyguanosin-8-yl)-IQ-3′-monophosphate adduct, an *N*-(deoxyguanosin-8-yl)-MeIQx-3′-monophosphate adduct, and an *N*-(deoxyguanosin-8-yl)-PhIP-3′-monophosphate adduct for IQ, MeIQx, and PhIP respectively, have all been found as principal adducts in *in vitro* assays using cell homogenates or by incubating reactive metabolites of these compounds with DNA (24, 27–29). An N^2-(deoxyguanosinyl)-MeIQx-3′-monophosphate adduct has also been identified by incubation of *N*-hydroxy-MeIQx with deoxyguanosine in the presence of acetic anhydride [27]. Other likely adducts of IQ, MeIQx, and PhIP are probably also derived from guanine, since incubations of DNA-reactive heterocyclic amine intermediates with deoxyadenosine, deoxycytosine, and deoxythymidine show no adducts by postlabeling (Fig. 7). These data suggest that the AIA heterocyclic amines prefer the C^8-position of guanine for adduction. The role of adduct structure on mutation and biological damage has yet to be determined for the AIA heterocyclic amines.

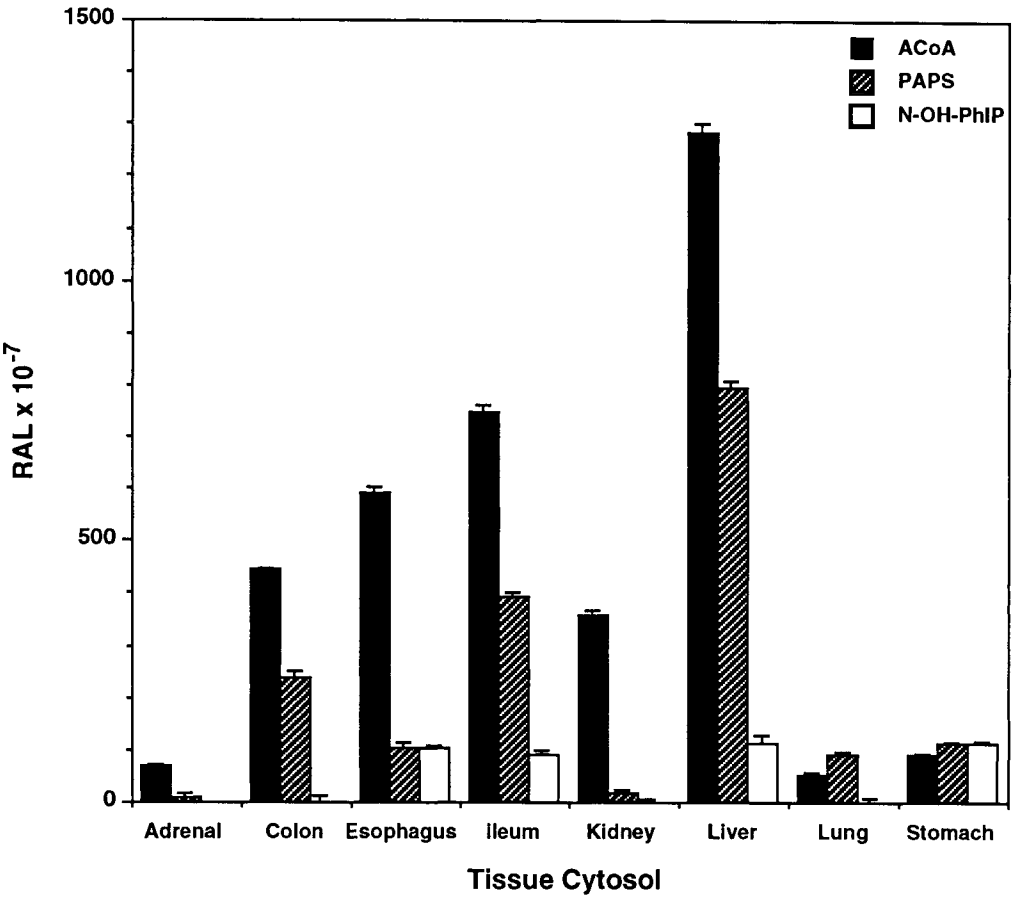

Fig. 6. Comparison of *N*:*O*-sulfation and *N*:*O*-acetylation of *N*-hydroxy-PhIP by human tissue cytosols. Assays were conducted as described [21].

CONCLUSIONS

DNA adducts are molecular lesions that develop early in the progression to cancer, a disease with a long latency. These biochemical changes are believed to be proximate causes of mutation and are critically involved in the mechanism of chemically induced cancer. DNA adducts have thus been suggested as potential markers of human carcinogen exposure and potential indicators of cancer risk. Adducts have also been used to study the metabolism of carcinogens to reactive intermediates using the DNA target as a trap for the unstable reactive metabolites. We have discussed the use of some very sensitive new tools in a number of model systems to help determine if dietary heterocyclic amines play a role in human cancer. We have also discussed the use of adducts to define the mechanisms by which heterocyclic amines lead to DNA adducts. These tools are now being applied to the study of other chemical carcinogens and the binding of carcinogens to protein.

DNA adduct dosimetry data with the heterocyclic amine MeIQx suggest that exposure dose is highly correlated with the biologically available dose and with the bioactive dose. Adduct levels decrease linearly with administered dose from the high doses used in cancer bioassays to relevant human exposure levels for all tissues we have studied in both single-dose 24 hour studies and sub-chronic 7-day exposures. Longer term chronic studies are underway. Comparison of adduction in different tissues with PhIP and MeIQx suggests that DNA adduct levels are tissue-dependent and that tumorigenesis in

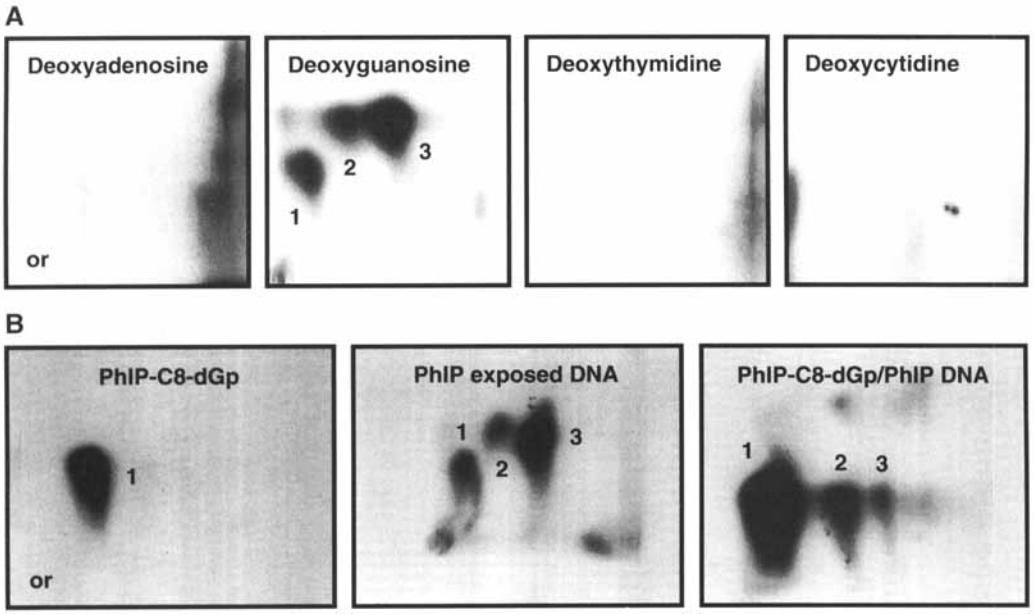

Fig. 7. ^{32}P-Postlabeling DNA adduct fingerprints for: (A) incubation of *N*-hydroxy-PhIP/acetic anhydride with deoxyadenosine-3'-monophosphate, deoxycytosine 3'-monophosphate, deoxyguanosine 3'-monophosphate, and deoxythymidine monophosphate. Adduct spots were seen only with deoxyguanosine. Similar results were found with MeIQx. (B) Demonstration that spot 1 from *in vivo* samples is the N^2-(2'-deoxyquanosin-8-yl)-PhIP adduct using authentic N^2-(2'-deoxyquanosin-8-yl)-PhIP (PhIP C^8-dGp), pancreatic DNA from a C57Bl/6 male mouse exposed to PhIP (20 mg/kg; PhIP-exposed DNA) and co-chromatography of the two (PhIP-C^8-dGp/PhIP DNA). ^{32}P-postlabeling was carried out as described [22]. Authentic N^2-(2'-deoxyquanosin-8-yl)-PhIP was kindly provided by Dr. H. Frandsen (National Food Agency of Denmark, Danish Ministry of Health).

target tissues is not necessarily correlated with adduct levels found in single-dose 24 hour studies or with biointoxification rates. Metabolism of the heterocyclic amines to DNA-damaging intermediates is somewhat compound-specific and, at least with PhIP, somewhat species-specific. Ultimately, metabolism and/or adduct repair rates among tissues and compounds may explain the lack of relationship between adducts and tumors for PhIP. Some of the adducts have been chemically identified, but the relationship between the adduct's structure and biological effect (mutation and tumors) are not presently known so risk estimates probably cannot be made as yet. What these data mean relative to determining human cancer risk from dietary exposure to heterocyclic amines remains to be determined. Since DNA damage by adduction is an early lesion that can begin the process of carcinogenesis, the linear dose-response suggests that adducts may be useful for identifying individuals receiving high exposure. Likewise, the relationship between metabolic capacity and adduct levels suggests that the AIA adduct levels may be somewhat useful in pinpointing individuals at high health risk when exposed to these compounds. However, much more work in assessing the role of specific adducts in mutation and tumorigenesis, as well as on DNA repair, needs to be carried out prior to linking biological outcome (*i.e.*, tumorigenesis) with molecular changes in the DNA and, ultimately, human cancer risk.

ACKNOWLEDGMENTS

This work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract number W-7405-ENG-48, and partially support-

ed by NIH grants CA55861-01 and ES04705-06. The authors wish to thank Jim Felton for helpful suggestions in conducting our research.

REFERENCES

1. Schulte P, Mazzuckelli LF: Validation of biological markers for quantitative risk assessment. Environ Health Persp 90:239–246, 1991.
2. Felton JS, Knize MG: Heterocyclic amine mutagens/carcinogens in foods. In Cooper CS, Grover PL (eds): "Handbook of Experimental Pharmacology." Berlin: Springer-Verlag, 1990, Vol 94/I, pp 471–502.
3. Vogel JS, Turteltaub KW: Bimolecular tracing through accelerator mass spectrometry. Trends in Analytical Chem 11:142–149, 1992.
4. Turteltaub KW, Felton JS, Gledhill BL, Vogel JS, Southon JR, Caffee MW, Finkel RC, Nelson DE, Proctor ID, Davis JC: Accelerator mass spectrometry in biomedical dosimetry: Relationship between low-level exposure and covalent binding of heterocyclic amine-carcinogens to DNA. Proc Natl Acad Sci, USA 87:5288–5292, 1990.
5. Randerath K, Randerath E: Monitoring carcinogen actions on DNA by ^{32}P-postlabeling. Int Symp Princess Takamatsu Cancer Res Fund 21:317–328, 1990.
6. Zhang X-M, Wakabayashi K, Liu Z-C, Sugimura T: Mutagenic and carcinogenic heterocyclic amines in Chinese foods. Mutat Res 201:181–188, 1988.
7. Felton JS, Knize MG: Mutagen formation in muscle meats and model heating systems. In Hayatsu H (ed): "Mutagens in Food: Detection and Prevention." 1990, pp 58–66.
8. Manabe S, Tohyama K, Wada O, Aramaki T: Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP), in cigarette smoke condensate. Carcinogenesis 12:1945–1947, 1991.
9. Peluso M, Castegnaro M, Malaveille C, Friesen M, Garren L, Hautefeuille A, Vineis P, Kadlubar F, Bartsch H: ^{32}P-postlabeling analysis of urinary mutagens from smokers of black tobacco implicates 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) as a major DNA-damaging agent. Carcinogenesis 12:713–717, 1991.
10. Felton JS, Knize MG, Shen NH, Andersen BD, Bjeldanes LF, Hatch FT: Identification of mutagens in cooked beef. Environ Health Persp, 67:17–24, 1986.
11. Sugimura T, Wakabayashi K: In Pariza MW (ed): "Mutagens and Carcinogens in Foods." New York: Wiley-Liss, 1990, pp 1–18.
12. Adamson RH, Thorgeirsson UP, Snyderwine EG, Thorgeirsson SS, Reeves J, Dalgard DW, Takayama S, Sugimura T: Carcinogenicity of 2-amino-3-methylimidazo(4,5-*f*)quinoline in non-human primates: Induction of tumors in three macaques. Jpn J Cancer Res 81:10–14, 1990.
13. Schut HAJ, Putman KOL, Randerath K: ^{32}P-Postlabeling analysis of DNA adducts in liver and small intestine of male Fischer-344 rats after intraperitoneal administration of 2-amino-3-methylimidazo-(4,5-*f*)quinoline (IQ). In King CM, Romano LJ, Schuetzle D (eds): "Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes." New York: Elsevier, 1987, pp 265–269.
14. Yamashita K, Umemoto A, Grivas S, Kato S, Sato S, Sugimura T: Heterocyclic amine-DNA adducts analyzed by ^{32}P-postlabeling method. Nucleic Acids Res 19:111–114, 1988.
15. Esumi H, Ohgaki H, Kohzen E, Takayama S, Sugimura T: Induction on 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.
16. Sugimura T, Sato S, Wakabayashi K: Mutagens-carcinogens in pyrolysates in amino acids and proteins and in cooked foods: Heterocyclic aromatic amines. In Woo Y-T, Lai DY, Arcos JC, Argus MF (eds): "Chemical Induction of Cancer: Structural Bases and Biological Mechanisms." San Diego, CA: Academic Press, Inc., 1988, pp 681–710.
17. Buonarati MH, Felton JS: Activation of 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) to mutagenic metabolites. Carcinogenesis 11:1133–1138, 1990.
18. Turteltaub KW, Knize MG, Buonarati MH, McManus ME, Veronese ME, Mazarimas JA, Felton JS: Metabolism of 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) by liver microsomes and isolated rabbit cytochrome P-450 isozymes. Carcinogenesis 11:941–946, 1990.
19. Wallin H, Mikalsen A, Guengerich FP, Ingelman-Sundberg M, Solberg KE, Rossland OJ, Alexander J: Differential rates of metabolic activation and detoxication of the food mutagen 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine by different cytochrome P-450 enzymes. Carcinogenesis 11:489–492, 1990.
20. Snyderwine EG, Roller PP, Adamson RH, Sato S, Thorgeirsson SS: Reaction of *N*-hydroxylamine and *N*-acetoxy derivatives of 2-amino-3-methylimidazo-(4,5-*f*)quinoline with DNA. Synthesis and identification of *N*-(deoxyguanosin-8-yl)-IQ. Carcinogenesis 9:1061–1065, 1988.
21. Buonarati MH, Turteltaub KW, Shen NH, Felton JS: Role of sulfation and acetylation in the activation of 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)-pyridine to intermediates which bind DNA. Mutat Res 140:61–65, 1990.
22. Turteltaub KW, Watkins BE, Vanderlaan M, Felton JS: Role of metabolism on the DNA binding of MeIQx in mice and bacteria. Carcinogenesis 11:43–49, 1990.
23. Asan E, Fasshauer I, Wild D, Denschler D: Heterocyclic aromatic amine-DNA adducts in bacterial and mammalian cells detected by ^{32}P-postlabeling analysis. Carcinogenesis 8:1569–1593, 1988.
24. Snyderwine EG, Wirth PJ, Roller PP, Adamson RH, Sato S, Thorgeirsson SS: Mutagenicity and *in vitro*

covalent binding of 2-hydroxyamino-3-methylimidazo(4,5-*b*)quinoline. Carcinogenesis 9:411–418, 1988.

25. McManus ME, Felton JS, Knize MG, Burgess WM, Roberts-Thompson S, Pond SM, Stupans I, Veronese ME: Activation of the food-derived mutagen 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine by rabbit and human liver microsomes and purified forms of cytochrome P-450. Carcinogenesis 10:357–363, 1989.
26. Shimada T, Guengerich FP: Activation of amino-alpha-carboline, 2-amino-1-methyl-6-phenylimidazo-(4,5-*b*)pyridine and a copper phthalocyanine cellulose extract of cigarette smoke condensate by cytochrome P-450 enzymes in rat and human microsomes. Cancer Res 51:5284–5291, 1991.
27. Turesky RJ, Rossi SC, Welti DH, Lay JO Jr, Kadlubar FF: Characterization of DNA adducts formed *in vitro* by reaction of *N*-hydroxy-2-amino-3-methylimidazo(4,5-*f*)quinoline and *N*-hydroxy-2-amino-3,8-dimethylimidazo(4,5-*f*)quinoxaline at the C^8 and N^2 atoms of guanine. Chem Res Toxicol 5:479–490, 1992.
28. Frandsen H, Grivas S, Andersson R, Dragsted L, Larsen JC: Reaction of the N^2-acetoxy derivative of 2-amino-1-methyl-6-phenyl(4,5-*b*)pyridine (PhIP) with 2′-deoxyguanosine and DNA. Synthesis and identification of N^2-(2′-deoxyguanosin-8-yl)-PhIP. Carcinogenesis 13:629–635, 1992.
29. Lin D, Kaderlik KR, Turesky RJ, Miller DW, Lay JO Jr, Kadlubar FF: Identification of *N*-(deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenyl(4,5-*b*)pyridine as the major adduct formed by the food-borne carcinogen, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)-pyridine, with DNA. Chem Res Toxicol 5:691–697, 1992.
30. McCoy EC, Anders M, Rosenkrantz HS: The basis of the insensitivity of the *Salmonella typhimurium* strain TA98/1,8DNP6 to the mutagenic action of nitroarenes. Mutat Res 121:17–23, 1983.
31. Maron DM, Ames BN: Revised methods for the *Salmonella* mutagenicity test. Mutat Res 113:173–215, 1983.