Published in final edited form as:

Chem Res Toxicol. 2011 June 20; 24(6): 913–925. doi:10.1021/tx200091y.

DNA Adduct Formation of 4-Aminobiphenyl and Heterocyclic Aromatic Amines in Human Hepatocytes

Gwendoline Nauwelaers[†], Erin E. Bessette[‡], Dan Gu[‡], Yijin Tang[‡], Julie Rageul[†], Valérie Fessard[§], Jian-Min Yuan^{||}, Mimi C. Yu^{||}, Sophie Langouët^{†,*}, and Robert J. Turesky^{‡,*}

[†] Institut de Recherche en Santé Environnement Travail, EA4427 SeRAIC, Université Rennes 1, IFR 140, 2 Avenue du Pr L Bernard, 35043 Rennes, France

[‡] Division of Environmental Health Science, Wadsworth Center, New York State Department of Health, Albany, New York 12201

[§] Anses Laboratoire de Fougères, La Haute Marche – Javené, BP 90203, 35302 Fougères cedex, France

^{||} Masonic Comprehensive Cancer Center, University of Minnesota, Minneapolis, MN 55455

Abstract

DNA adduct formation of the aromatic amine, 4-aminobiphenyl (4-ABP), a known human carcinogen present in tobacco smoke, and the heterocyclic aromatic amines (HAAs), 2-amino-9H-pyrido[2,3-*b*]indole (AαC), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), potential human carcinogens, which are also present in tobacco smoke or formed during the high-temperature cooking of meats, was investigated in freshly cultured human hepatocytes. The carcinogens (10 μM) were incubated with hepatocytes derived from eight different donors for time periods up to 24 h. The DNA adducts were quantified by liquid chromatography-electrospray ionization mass spectrometry with a linear quadrupole ion trap mass spectrometer. The principal DNA adducts formed for all of the carcinogens were *N*-(deoxyguanosin-8-yl) (dG-C8) adducts. The levels of adducts ranged from 3.4 to 140 adducts per 10⁷ DNA bases. The highest level of adduct formation occurred with AαC, followed by 4-ABP, then by PhIP, MeIQx, and IQ. Human hepatocytes formed dG-C8-HAA-adducts at levels that were up to 100-fold greater than the amounts of adducts produced in rat hepatocytes. In contrast to HAA adducts, the levels of dG-C8-4-ABP adduct formation were similar in human and rat hepatocytes. These DNA binding data demonstrate that the rat, an animal model that is used for carcinogenesis bioassays, significantly underestimates the potential hepatic genotoxicity of HAAs in humans. The high level of DNA adducts formed by AαC, a carcinogen produced in tobacco smoke at levels that are up to 100-fold higher than the amounts of 4-ABP, is noteworthy. The possible causal role of AαC in tobacco-associated cancers warrants investigation.

INTRODUCTION

4-Aminobiphenyl (4-ABP) is a prototypical aromatic amine and a recognized human urinary bladder carcinogen (1–3). 4-ABP served as an antioxidant in the rubber industry prior to

sophie.langouet@univ-rennes1.fr; Tel. 02 23 23 48 06 Fax: 02 23 23 47 94. Rturesky@wadsworth.org; Tel. 518-474-4151; Fax 518-473-2095.

*These authors contributed equally to this work

SUPPORTING INFORMATION PARAGRAPH

Additional information as noted in text. This material is available free of charge via the Internet at <http://pubs.acs.org>.

discovery of its carcinogenic activity (2,3). In the United States and many developed countries, strict federal regulations have drastically diminished the industrial usage of 4-ABP and other carcinogenic aromatic amines, but 4-ABP can still be found as a contaminant in color additives (4,5), paints (6), food colors (7), leather and textile dyes (8,9). 4-ABP and other aromatic amines also arise in diesel-exhaust particles (10–12) and cooking oil fumes (13). A major source of exposure to 4-ABP occurs through tobacco smoke: 4-ABP is present in mainstream tobacco smoke at levels ranging from 0.1 to 4.3 ng/cigarette (14,15). Another potential source of exposure to 4-ABP occurs through the usage of commercial hair dyes (16,17).

Heterocyclic aromatic amines (HAAs) are carcinogens in experimental laboratory animals and potential human carcinogens (18). HAAs are structurally related to aromatic amines: both classes of compounds undergo common pathways of bioactivation (19,20). The principal source of exposure to HAAs occurs through consumption of well-done cooked meats: the concentrations of HAAs can range from <1 part-per-billion up to the 500 ppb level (18,21,22). Several HAAs are also formed during the high-temperature burning of tobacco. 2-Amino-9*H*-pyrido[2,3-*b*]indole (AαC) was one of the first chemicals in a series of HAAs identified in protein pyrolysates (23,24). Thereafter, AαC was detected in mainstream tobacco smoke at levels ranging from 60 to 258 ng/cigarette (25,26). These levels are ~25 to 100-fold higher than the levels of 4-ABP, 2-naphthylamine, or benzo(a)pyrene (27) present in mainstream smoke and are comparable to the levels of tobacco specific nitrosamines, such as 4-(methyl-nitrosamino)-1-(3-pyridyl)-1-butanone (28). The latter compounds are known human carcinogens. 2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) occurs in mainstream tobacco smoke at levels that range from 11 to 23 ng/cigarette (29). PhIP also forms during the combustion of organic material, and it is present in air-borne and diesel exhaust particles (12). 2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ) was reported at levels below 1 ng/cigarette (30), whereas 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) was not detected. Thus, there is much concern about the health risk associated with the exposure to these structurally related class of chemicals found in tobacco smoke and cooked meats.

One approach that has been used to assess the carcinogenic potential of genotoxic chemicals is the carcinogen binding index (31). This approach has been used in rodent models, where the DNA adduct levels of carcinogens measured in liver at the given dose are normalized to the dose which resulted in a 50% tumor incidence under the conditions of a 2-year bioassay (TD₅₀ dose). The carcinogen binding index has been used as a preliminary assessment of the carcinogenic potential of a chemical (31); however, the development of cancer is a multifactorial process, that requires a number of steps, including mutations, genetic alterations, and cell proliferation (18). Moreover, the extrapolation of animal carcinogenicity data to assess human health risk has many uncertainties, which can lead to tenuous risk assessment estimates. Perhaps the most critical and variable parameters in interspecies extrapolation are the effects of dose, species differences in catalytic activities of xenobiotic metabolism enzymes, and the influence of genetic polymorphisms that encode enzymes that lead to interindividual differences in metabolism of carcinogens (32).

The liver is the most metabolically active organ in the biotransformation of 4-ABP and HAAs to genotoxic metabolites (33,34). We have previously shown that freshly isolated human hepatocytes in primary culture effectively metabolize a variety of chemical carcinogens (35), including aflatoxin B₁ (36), MeIQx (37), and PhIP (38) to reactive metabolites capable of damaging DNA. In this study, we have investigated the capacity of human and rat hepatocytes to bioactivate 4-ABP and several HAAs, to form DNA adducts, which are believed to be the initiating events of chemical carcinogenesis (39). Our data show that HAAs covalently bind to DNA in human hepatocytes at considerable levels.

Moreover, the levels of AαC adducts formed are comparable to the levels of DNA adducts formed with 4-ABP, a known human carcinogen. The amounts of HAA-DNA adducts formed in human hepatocytes are much greater than the levels of adducts produced in hepatocytes of rats, a species that is used in long-term carcinogen bioassays and extrapolation of toxicity data for human risk assessment. Thus, the data on the carcinogen binding index of HAAs in rat hepatocytes greatly underestimates the genotoxic potential of HAAs in human hepatocytes.

Materials and Methods

Caution

AαC, 4-ABP, IQ, MeIQx, PhIP are carcinogens, and they should only be handled in a well-ventilated fume hood with the appropriate protective clothing.

Chemicals

MeIQx, 3-[²H₃C]-MeIQx, IQ, 3-[²H₃C]-IQ and PhIP, and 1-[²H₃C]-PhIP, were purchased from Toronto Research Chemicals (Toronto, ON, Canada). AαC and [¹³C₆]-AαC were kindly provided by Dr. Dan Doerge NCTR (Jefferson, AR). The isotopic purities of all compounds exceeded 99.5%, except for 3-[²H₃C]-MeIQx, which was 96.5% isotopically pure. 4-ABP was purchased from Aldrich (Milwaukee, WI). 2-Nitro-9H-pyrido[4,5-*b*]indole was a kind gift from Dr. D. Miller, NCTR (Jefferson, AR). [²H₉]-4-Nitrobiphenyl (99.3% isotopically pure) was purchased from C/D/N Isotopes, Inc. (Pointe-Claire, Quebec, Canada). [²H₉]-4-ABP was prepared by reduction of [²H₉]-4-nitrobiphenyl with Zn in C₂H₅OH/HCl at 60 °C for 2 h as previously described (16). [¹³C₁₀]-dG (99.9% isotopically pure) was purchased from Cambridge Isotopes (Andover, MA). Alkaline phosphatase (from *E. coli*), and nuclease P1 (from *Penicillium citrinum*) were purchased from Sigma (St. Louis, MO). Phosphodiesterase I (from *Crotalus adamanteus* venom) was from GE Healthcare (Piscataway, NJ). All solvents used were high-purity B & J Brand from Honeywell Burdick and Jackson (Muskegon, MI). ACS reagent-grade formic acid (88%) was purchased from J.T. Baker (Phillipsburg, NJ). HyperSep™ filter SpinTips C18 (20 mg) were from Thermo Scientific (Palm Beach, FL). Oragene-DNA saliva kits were from Genotek Inc. (Ontario, Canada).

Preparation of MeIQx and PhIP metabolites

The following metabolites and their trideuterated internal standards were prepared by chemical means (37) or biosynthetically as previously reported (40): 2-Amino-3-methylimidazo[4,5-*f*]quinoxaline-8-carboxylic acid (IQx-8-COOH); 2-amino-8-(hydroxymethyl)-3-methylimidazo[4,5-*f*]quinoxaline (8-CH₂OH-IQx); *N*²-(β-1-glucosiduronyl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx-*N*²-Gl); *N*²-(β-1-glucosiduronyl)-2-(hydroxyamino)-3,8-dimethylimidazo[4,5-*f*]quinoxaline (HON-MeIQx-*N*²-Gl), *N*²-(3,8-dimethylimidazo[4,5-*f*]quinoxalin-2-yl-sulfamic acid (MeIQx-*N*²-SO₃H); *N*²-(β-1-glucosiduronyl)-2-(hydroxyamino)-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (HON-PhIP-*N*²-Gl); *N*³-(β-1-glucosiduronyl)-2-(hydroxyamino)-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (HON-PhIP-*N*³-Gl); *N*²-(β-1-glucosiduronyl)-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP-*N*²-Gl); and *N*³-(β-1-glucosiduronyl)-2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP-*N*³-Gl).

Preparation of the DNA adducts

N-(Deoxyguanosin-8-yl)-AαC (dG-C8-AαC) (41), *N*-(deoxyguanosin-8-yl)-IQ (dG-C8-IQ), *N*-(deoxyguanosin-8-yl)-IQ, 5-(deoxyguanosin-*N*²-yl)-2-amino-3-methylimidazo[4,5-*f*]quinoxaline (dG-*N*²-IQ), *N*-(deoxyguanosin-8-yl)-MeIQx (dG-C8-MeIQx), and 5-

(deoxyguanosin-*N*²-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (dG-*N*²-MeIQx) (42,43), and *N*-(deoxyguanosin-8-yl)-PhIP (dG-C8-PhIP) (44,45) were prepared by reaction of their *N*-acetoxy-HAA derivatives with dG or [¹³C₁₀]-dG (5 mg) in 100 mM potassium phosphate buffer (pH 8.0). *N*-(Deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-4-ABP) was prepared by reaction of *N*-hydroxy-4-ABP with pyruvonnitrile, followed by reaction with dG or [¹³C₁₀]-dG (46). For the case of dG-C8-MeIQx, the internal standard was prepared by reaction of the *N*-acetoxy derivative of 3-[²H₃C]-MeIQx with dG. The adducts were purified by solid phase extraction, followed by HPLC purification (42,43).

Cell Isolation, Culture, and Metabolism Studies

Human liver samples were obtained from eight patients undergoing liver resection for primary or secondary hepatomas through the Biological Resource Center (CHRU Pontchaillou, Rennes, France). The research protocol was conducted under French legal guidelines and fulfilled the requirements of the local institutional ethics committee. This study was approved by the Institutional Review Board at the Wadsworth Center. Hepatocytes were isolated by a two-step collagenase perfusion procedure. The liver parenchymal cells were seeded in Petri dishes at a density of 3×10^6 viable cells/19.5 cm² dish, in 3 mL of Williams' medium with the following supplements: bovine serum albumin (1 g/L), glutamine (2 mM), bovine insulin (5 μg/mL), penicillin (10 U/mL), streptomycin (10 μg/mL) and fetal calf serum (10% v/v). The cell culture medium was renewed daily. After 36 h cell seeding, the media was replenished with cell media lacking fetal calf serum and hydrocortisone hemisuccinate (54 μM) was added. Then, 4-ABP or HAAs (10 μM) in DMSO (0.1% v/v) was added to the medium, and the cells were incubated for 3, 8 or 24 h. The incubations were terminated by collecting the culture medium. Adult male Sprague Dawley rats (200–250 g) were purchased from Janvier (Genest-Saint-Isle, France). Liver parenchymal cells were prepared and seeded at the same density, and treated with carcinogens as described above. No toxicity was observed with any compounds on the basis of the methylthiazoltetrazolium test for either human or rat hepatocytes.

The cells were scraped from the P60 Petri dishes with a spatula and combined with the cell media in Eppendorf tubes, followed by centrifugation. The supernatant containing the cell culture media was retrieved from the cellular pellets containing DNA, and both fractions were stored at –80 °C, prior to analyses. The cell culture media (5 μL) containing the equivalent of 11.2 ng of PhIP and its metabolites (50 pmol of PhIP) or the equivalent of 10.7 ng of MeIQx and its metabolites (50 pmol of MeIQx) were diluted with water (45 μL), followed by the addition of 750 pg of isotopically labeled internal standard for each metabolite of PhIP or MeIQx and either 250 pg of 3-[²H₃C]-MeIQx or 1-[²H₃C]-PhIP. The cell culture media of hepatocyte samples of 4-ABP, AαC and IQ (5 μL, 50 pmol of carcinogen) were diluted with water (45 μL), and were spiked with 250 pg of [²H₉]-4-ABP, 3-[²H₃C]-IQ, or [¹³C₆]-AαC. The samples were added to CH₃OH (150 μL) and incubated on ice for 1 h, to promote precipitation of salts and protein. The samples were then centrifuged at 15000 g for 3 minutes at 4 °C, and the supernatants were transferred to capLC recovery vials and concentrated to dryness by vacuum centrifugation. The extracts were dissolved in water and assayed by LC-ESI/MS/MS (*vide infra*).

Ethoxyresorufin *O*-deethylation (EROD) associated with P450 1A1/2 activity was measured in cultured hepatocytes essentially as described by Burke and Mayer (47). Methoxyresorufin *O*-demethylation (MROD) was used as a substrate for specific P450 1A2 activity (48). The reaction rates were linear with time and proportional to protein concentration. Cellular protein content was estimated by the Bradford procedure (49). Values (pmol/min/mg protein) are the mean ± SD of quadruplicate measurements.

Isolation and Enzymatic Digestion of DNA, and Solid Phase Enrichment of DNA Adducts

DNA was isolated from the cellular pellets by a chloroform/phenol extraction method (50). The amount of DNA recovered from each carcinogen treatment was determined by UV spectroscopy, assuming a concentration of DNA ($50 \mu\text{g/mL}$) is equal to 1.0 absorbance unit at 260 nm. Isotopically labeled internal standards of each DNA adduct were added to the DNA recovered from the treated hepatocytes at a level of 1 adduct per 10^6 DNA bases. The DNA from the respective time points of the individually treated 4-ABP and HAA hepatocyte samples were then pooled. The enzymatic digestion conditions used for the hydrolysis of DNA ($\sim 2 - 10 \mu\text{g}$) in 5 mM Bis-Tris-HCl buffer (pH 7.1, $50 \mu\text{L}$) employed DNase I for 1.5 h, followed by incubation with nuclease P1 for 3 h, and then digested with alkaline phosphatase and phosphodiesterase for 18 h (45). These enzyme digestion conditions were shown to be highly efficient in the recovery of the dG-C8 adducts of PhIP, MeIQx, IQ, and 4-ABP from calf thymus DNA modified with these carcinogens (42–45,51,52). The DNA adducts were purified by solid phase extraction, using HyperSepTM filter SpinTips, as previously described (52,53).

LC-ESI-MS/MS Measurements for Metabolites—The analyses of 4-ABP and HAAs, and the metabolites of MeIQx and PhIP were performed with a NanoAcquityTM UPLC system (Waters Corporation, Milford, MA) equipped with a Michrom C18 AQ column ($0.3 \times 150 \text{ mm}$, $3 \mu\text{m}$ particle size, Michrom Bioresources Inc., Auburn, CA), as previously described (54). The analytes were separated by a gradient. The A solvent was 0.01% HCO_2H in H_2O , and the B solvent contained 0.01% HCO_2H and 5% H_2O in CH_3CN . The flow rate was set at $6 \mu\text{L/min}$, starting at 100% A increased by a linear gradient to 65% B in 13 min, and then to 100% B at 14 min holding for 1 min. The gradient was reversed to the 100% A over 1 min and at the same time the flow rate was increased from $6 \mu\text{L/min}$ to $10 \mu\text{L/min}$. A post-run time of 3 min was required for re-equilibration. The back pressure of the column was 3,700 psi at the initial solvent conditions. The sample injection volume was $0.4 \mu\text{L}$. The manipulation of UPLC system was done by MassLynx software (Waters Corp., Milford, MA).

The mass-spectral data were acquired on a FinniganTM Quantum Ultra Triple Stage Quadrupole MS (Thermo Fisher, San Jose, CA) and processed with Xcalibur version 2.07 software. Analyses were conducted in the positive ionization mode and employed an Advance CaptiveSprayTM source from Michrom Bioresources Inc. (Auburn, CA). The spray voltage was set at 1400 V; the in-source fragmentation was 5 V; and the capillary temperature was 200°C . There was no sheath or auxiliary gas. The peak widths (in Q1 and Q3) were set at 0.7 Da. The measurement of analytes was done by selected reaction monitoring (SRM). The following transitions and collision energies were used for the quantification of MeIQx, PhIP, and their metabolites: MeIQx and $[\text{}^2\text{H}_3\text{C}]\text{-MeIQx}$: $214.1 \rightarrow 199.1$ and $217.1 \rightarrow 199.1$, at 30 eV; 8- CH_2OH -IQx and $[\text{}^2\text{H}_3\text{C}]\text{-8-CH}_2\text{OH-IQx}$: $230.1 \rightarrow 197.1$ and $233.1 \rightarrow 197.1$, at 35 eV; IQx-8-COOH and $[\text{}^2\text{H}_3\text{C}]\text{-IQx-8-COOH}$: $244.1 \rightarrow 183.1$ and $247.1 \rightarrow 183.1$, at 38 eV; MeIQx- N^2 - SO_3H and $[\text{}^2\text{H}_3\text{C}]\text{-MeIQx-}N^2\text{-SO}_3\text{H}$: $294.1 \rightarrow 214.1$ and $297.1 \rightarrow 217.1$ @ 35 eV; MeIQx- N^2 -Gl and $[\text{}^2\text{H}_3\text{C}]\text{-MeIQx-}N^2\text{-Gl}$: $390.1 \rightarrow 214.1$, 212.1 , 199.1 and $393.1 \rightarrow 217.1$, 215.1 , 199.1 @ 55 eV; HON-MeIQx- N^2 -Gl and $[\text{}^2\text{H}_3\text{C}]\text{-HON-MeIQx-}N^2\text{-Gl}$: $406.1 \rightarrow 214.1$, 213.1 , 212.1 and $409.1 \rightarrow 217.1$, 216.1 , 215.1 @ 35 eV; PhIP and $[\text{}^2\text{H}_3\text{C}]\text{-PhIP}$: $225.1 \rightarrow 210.1$ and $228.1 \rightarrow 210.1$ at 33 eV; PhIP- N^2 -Gl, PhIP- N^3 -Gl and $[\text{}^2\text{H}_3\text{C}]\text{-PhIP-}N^2\text{-Gl}$, $[\text{}^2\text{H}_3\text{C}]\text{-PhIP-}N^3\text{-Gl}$: $401.1 \rightarrow 210.1$ and $404.1 \rightarrow 210.1$ at 55 eV; HON-PhIP- N^2 -Gl, and $[\text{}^2\text{H}_3\text{C}]\text{-HON-PhIP-}N^2\text{-Gl}$: $417.1 \rightarrow 225.1$ and 224.1 and $420.1 \rightarrow 228.1$ and 227.1 @ 34 eV; HON-PhIP- N^3 -Gl and $[\text{}^2\text{H}_3\text{C}]\text{-HON-PhIP-}N^3\text{-Gl}$: $417.1 \rightarrow 225.1$ and 224.1 and $420.1 \rightarrow 228.1$ and 227.1 @ 34 eV. The transitions employed for IQ and $[\text{}^2\text{H}_3\text{C}]\text{-IQ}$ were $199.1 \rightarrow 184.1$ and $202.1 \rightarrow 184.1$ @ 32 eV; the transitions of A α C and $[\text{}^{13}\text{C}_6]\text{-A}\alpha\text{C}$ were $184.1 \rightarrow 167.1$ and $190.1 \rightarrow 173.1$ @ 20 eV; and the transitions

employed for 4-ABP and [$^2\text{H}_9$]-4-ABP were 170.1 $\rightarrow$ 153.1 and 179.1 $\rightarrow$ 160.1 @ 30 eV. The dwell time for each transition was 10 ms. Argon was used as the collision gas and was set at 1.5 mTorr. Product ion spectra were acquired on the protonated molecules $[\text{M} + \text{H}]^+$, scanning from m/z 50 to 250 or 500 at a scan speed of 500 amu/s using the same acquisition parameters as above.

LC-ESI/MS/MS³ Measurements for DNA Adducts—The DNA adduct analyses were conducted with an Agilent 1100 Series capillary LC system (Agilent Technologies, Palo Alto, CA) equipped with an Aquasil C18 column (0.32 $\times$ 250 mm) from Thermo Fisher (Bellafonte, PA). Samples (2 μL) were injected, and analytes were separated with a gradient. The solvent conditions were held at 100% A (solvent composition: 0.01% HCO_2H and 10% CH_3CN) for 2 min, followed by a linear gradient to 100% B (solvent composition: 95% CH_3CN containing 0.01% HCO_2H) over 30 min at a flow rate of 6 $\mu\text{L}/\text{min}$. The MS instrumentation was a linear quadrupole ion trap mass spectrometer (LTQ, Thermo Fisher, San Jose, CA), and Xcalibur version 2.07 software was used for data manipulations. Analyses were conducted in the positive ionization mode and employed an Advance CaptiveSprayTM source from Michrom Bioresource Inc. (Auburn, CA). Representative optimized instrument tuning parameters were as follows: capillary temperature 220 $^\circ\text{C}$; source spray voltage 1.5 kV; source current 0.3 μA ; no sheath gas, sweep gas or auxiliary gas was employed; capillary voltage 32 V; tube lens voltage 110 V; and in-source fragmentation 10 V.

The LTQ MS was employed in the tandem MS/MS scan mode to monitor the loss of deoxyribose from the protonated molecules of the adducts ($[\text{M} + \text{H} - 116]^+$), followed by the consecutive reaction monitoring scan mode at the MS³ scan stage, to characterize the product ions of the aglycone adducts $[\text{BH}_2]^+$ (45,53,55). The total ion counts produced at the MS³ scan stage were used for quantitative measurements for all of the adducts except for dG-C8-IQ and [$^{13}\text{C}_{10}$]-dG-C8-IQ. The ions monitored in MS $\rightarrow$ MS² $\rightarrow$ scan modes were as follows: dG-C8-PhIP (m/z 490.1 $\rightarrow$ 374.1); [$^{13}\text{C}_{10}$]-dG-C8-PhIP (m/z 500.1 $\rightarrow$ 379.1); dG-C8-MeIQx (m/z 479.1 $\rightarrow$ 363.1); dG-C8- $[\text{H}_3\text{C}]$ -C8-MeIQx (m/z 482.1 $\rightarrow$ 366.1); dG-A α C (m/z 449.1 $\rightarrow$ 333.1); [$^{13}\text{C}_{10}$]-dG-A α C (m/z 459.1 $\rightarrow$ 338.1); dG-C8-4-ABP (m/z 435.1 $\rightarrow$ 319.1); [$^{13}\text{C}_{10}$]-dG-C8-4-ABP (m/z 445.1 $\rightarrow$ 324.1), dG-C8-IQ (m/z 464.1 $\rightarrow$ 348.1); [$^{13}\text{C}_{10}$]-dG-C8-IQ (m/z 474.1 $\rightarrow$ 353.1). Isobaric interferences precluded the use of total ion counts for measurement of dG-C8-IQ and ([$^{13}\text{C}_{10}$]-dG-C8-IQ; the extracted ions at m/z 302.2 and 331.1, and 307.2 and 336.2, respectively, produced at the MS³ scan stage, were used for quantitative measurements. The [$^{13}\text{C}_{10}$]-dG-C8-4-ABP, [$^{13}\text{C}_{10}$]-dG-C8-IQ and [$^{13}\text{C}_{10}$]-dG-C8-MeIQx were employed as internal standards to estimate the levels of dG- N^2 - N^4 -ABP, dG- N^2 -IQ and dG- N^2 -MeIQx.

The normalized collision energies were set at 32 and 40, and the isolation widths were set at 3.0 and 1.0 Da, respectively, for the MS² and MS³ scan modes, for all adducts. The activation Q was set at 0.35 and the activation time was 10 ms, for both scan modes. Helium was used as the collision damping gas in the ion trap and was set at a pressure of 1 mTorr. One μscan was used for data acquisition. The automatic gain control settings were full MS target 30,000 and MSⁿ target 10,000, and the maximum injection time was 10 ms.

Calibration curves—Estimates of MeIQx and PhIP metabolites were done with external calibration curves using 50 pg of isotopically labeled parent compounds and 150 pg of isotopically labeled metabolites. The unlabeled metabolite calibrant levels ranged from 0.5 to 30-fold the amount of the internal standards. The calibration curves were fitted to a straight line (area of response of the metabolite/internal standard versus the amount of metabolite/internal standard) using ordinary least-squares with equal weightings. The coefficient of determination (r^2) values of the slopes exceeded 0.99.

Calibration curves were constructed with the stable, isotopically labeled DNA adducts set at 14.3 pg and a 6 point calibration curve with unlabeled DNA adducts was established with 0, 1.43 pg - 143 pg of each adduct added to the DNA digest matrix (10 μ g), following solid phase extraction. Assuming an average recovery of 10 μ g of DNA (30 nmol of deoxynucleoside) from each hepatocyte incubation, the level of internal standard represented 10 adducts per 10^7 bases and the levels of unlabeled adduct ranged from 1 to 100 adducts per 10^7 bases. The calibration curves were done in triplicate at each level, and the data were fitted to a straight line (area of response of the adduct/internal standard versus the amount of adduct/internal standard) using ordinary least-squares with equal weightings. The coefficient of determination (r^2) values of the slopes exceeded 0.98.

Results

4-ABP and HAA Metabolism in Human Hepatocytes

4-ABP, A α C, IQ, MeIQx, and PhIP undergo metabolic activation at the exocyclic amine group by cytochrome P450 enzymes to produce the genotoxic *N*-hydroxylated metabolites. These intermediates undergo esterification by phase II enzymes, including *N*-acetyltransferases (NATs) or sulfotransferases (SULTs), to produce electrophiles that react with DNA (19,20,56–58). The structures of these carcinogens and their DNA adducts are shown in Figure 1.

The kinetics of metabolism and DNA adduct formation of the carcinogens were determined following 3, 8 or 24 h of incubation. These time points were selected on the basis of our previous studies on PhIP and MeIQx (10 μ M) with human hepatocytes, which showed that up to 97% of the doses underwent metabolism after 24 h incubation. The extent of metabolism was strongly correlated to the level of P4501A2 enzyme activity (37,38). The rates of metabolism of HAAs and 4-ABP were measured in hepatocyte preparations from two donors. The metabolism of HAAs was more rapid than that of 4-ABP (Figure 2). At the 24 h time point, 71 – 98% of the doses of HAAs underwent metabolism, whereas 59 to 77% of the amount of 4-ABP underwent biotransformation. *N*-hydroxy-4-ABP was reported to undergo reduction to form 4-ABP at greater rates than the *N*-hydroxy-HAA substrates formed the parent HAAs, by an NADPH-dependent reductase, when employing rat or human microsomes, rat hepatocytes or human HepG2 cells (59). This reductase activity could influence the relative amounts of 4-ABP or HAAs remaining in primary culture.

We previously characterized the major pathways of metabolism of MeIQx and PhIP in freshly cultured human and rat hepatocytes employing the 14 C-labelled HAAs as substrates (37,38). In this current study, we measured the amounts of the principal metabolites of MeIQx and PhIP produced by human hepatocytes, employing LC-ESI/MS/MS with stable, isotopically labeled internal standards for quantitative measurements. The metabolic pathways of MeIQx and PhIP are depicted in Figure 3, and the kinetics of metabolite formation are shown in Figure 4. The LC-ESI/MS/MS traces of MeIQx and PhIP metabolites (formed at T 24 h) and their product ion spectra are shown in Supporting Information, Figures S-1 and S-2. The findings on metabolite formation are consistent with our earlier studies that employed radiolabelled substrates (37,38). Both MeIQx and PhIP undergo *N*-oxidation by P450 1A2, to produce HONH-MeIQx and HONH-PhIP as principal oxidation products. These *N*-hydroxylated HAAs undergo conjugation with glucuronic acid to form HON-MeIQx-*N*²-Gl and HON-PhIP-*N*²-Gl. The isomeric glucuronide conjugate HONH-*N*3-PhIP is formed, but it occurs at ~10-fold lower levels than HON-PhIP-*N*²-Gl. P450 1A2 also mediates oxidation of the 8-CH₃ group of MeIQx, to produce the alcohol IQx-8-CH₂OH and the carboxylic acid, IQx-8-COOH, as other prominent metabolites (60). The phase II conjugation products MeIQx-*N*²-SO₃H, MeIQx-*N*²-Gl and the isomeric *N*-glucuronide conjugates PhIP-*N*²-Gl and PhIP-*N*3-Gl were also formed, the latter PhIP-*N*-Gl

metabolites were produced at considerably lower levels than the HON-PhIP-*N*-Gl metabolites.

We have also examined the metabolism of AαC in human hepatocytes, by LC-ESI/MS/MS, in positive and negative ionization modes (Supporting Information, Figure S-3 A and Figure S-3 B). There is evidence for the formation of two glucuronide conjugates of AαC; four glucuronide conjugates of ring- or N-oxidized AαC; and two sulfate conjugates of AαC. The elucidation of the sites of AαC oxidation and phase II conjugation requires further study.

Monitoring 4-ABP and HAA DNA Adduct Formation in Human Hepatocytes

The LTQ MS was employed at the MS³ stage, to characterize and measure product ions of the aglycone adducts [BH₂]⁺. The LC-ESI/MS/MS³ traces of DNA adducts formed in hepatocytes treated with carcinogens for 8 h are shown in Figure 5. The dG-C8 DNA adducts of all carcinogens were readily identified. In addition, an isomeric dG-*N*² adduct of 4-ABP (tentatively assigned) and a minor 4-ABP adduct formed at the C8 atom with dA were detected (55,56). The dG-*N*² adducts of MeIQx and IQ were also identified. The LC-ESI/MS/MS³ product ion spectra of the DNA adducts and the respective internal standards are shown in Supporting Information, Figure S-4. The product ion spectra provide rich structural information about the structures of the adducts and corroborate their identities (45,52,53,58).

The kinetics of DNA adduct formation were investigated in human hepatocytes of 4 donors (Figure 6), including donors F and G, which were examined for the metabolism of MeIQx and PhIP. At the earliest time point measured (3 h), the amount of the dG-C8-AαC formed was far greater than the levels of DNA adducts formed by any of the other carcinogens. The levels of dG-C8-HAA adduct formation peaked between 3 and 8 h of incubation, whereas the amount of dG-C8-4-ABP adduct formation continued to increase during the course of the 24 h incubation, perhaps reflective of a slower rate of uptake or metabolism of 4-ABP in comparison to the HAAs (Figure 2). The kinetics of DNA adduct formation were similar in the hepatocytes of the 4 donors. The levels of dG-C8-HAA and dG-C8-4-ABP adduct formation at 24 h for the 8 donors are reported in Table 1. The highest levels of adducts were consistently formed by AαC and 4-ABP, followed by PhIP, MeIQx and IQ.

4-ABP and HAA DNA Adduct Formation in Rat Hepatocytes

The kinetics of 4-ABP- and HAA-DNA adduct formation were also determined in hepatocytes isolated from 3 different rats (Figure 7). The EROD activities ranged from 0.4 to 2.7 pmol resorufin/min/mg protein, and MROD activities ranged from 0.8 to 1.6 pmol resorufin/min/mg protein. These P450 enzyme activities were comparable, if not superior to the P450 enzyme activities measured in the hepatocytes of 3 human donors (Table 1).

The amounts of dG-C8-4-ABP ranged from 4.2 to 7.2 adducts per 10⁶ bases following 24 h of incubation; these levels of adduct formation are similar to the amounts of dG-C8-4-ABP formed in human hepatocytes (Figure 4). dG-C8-AαC was the second most abundant adduct formed: the levels ranged from 0.5 to 3.1 adducts per 10⁶ bases at 24 h of incubation, followed by dG-C8-MeQx (0.1 – 0.3 adducts per 10⁶ bases/24 h), and then by dG-C8-PhIP (0.01 – 0.02 adducts per 10⁶ bases/24 h).

Discussion

Human hepatocytes are highly efficient in the bioactivation of 4-ABP and HAA and in ensuing DNA adduct formation. The highest levels of adducts were formed with AαC, followed by 4-ABP, with lesser but appreciable amounts of adducts formed with PhIP, MeIQx, and IQ. 4-ABP induces cancer of the urinary bladder, liver, and mammary gland in

rodents (61,62), and 4-ABP is a recognized human urinary bladder carcinogen (2). Cigarette smokers are at elevated risk for urinary bladder cancer, and the role of aromatic amines, including 4-ABP, as causal agents of this disease is well documented (2,63,64). It is noteworthy that the amounts of AαC present in cigarette smoke are far greater than the levels of 4-ABP: up to 258 ng AαC is produced in mainstream smoke per cigarette versus 0.1 to 4.3 ng 4-ABP/cigarette (14,15). AαC causes liver and blood vessel tumors in CDF₁ mice (18). AαC is a potent *lacI* transgene mutagen in the colon of C57BL/6 mice (Big Blue mouse) (65), and it induces aberrant crypt foci, early biomarkers of tumors, in the colon of this species (66); although carcinogen bioassays with AαC have not been conducted in this mouse strain. We note that even though AαC forms DNA adducts at high levels in rat hepatocytes (Figure 6), AαC is not a hepatocarcinogen in the rat model (18). HAAs and other genotoxicants form adducts in a number of tissues of experimental animals, where tumors do not develop during long-term feeding studies (67). The development of cancer is complex and requires a number of steps, including multiple mutations, genetic alterations, and cell proliferation (18,68). AαC present in tobacco smoke may act as an initiating agent in the development of human cancer during chronic smoking, in which many other mutagens-carcinogens, tumor promoters, and factors stimulating tumor progression exist (18).

The global DNA adduct formation and the genotoxicity of MeIQx, PhIP, AαC and 4-ABP in the hypoxanthine phosphoribosyl transferase (*hprt*) gene of Chinese hamster ovary cells (CHO) stably transfected with *P450 1A1* or *P450 1A2* and either *NAT2*4* or *NAT2*5B* alleles were recently examined (53,69–71). On the basis of estimates of dG-C8-HAA and dG-C8-4-ABP adduct formation and assuming that these adducts are the principal genotoxic lesions, we estimate that one dG-C8-HAA or dG-C8-4-ABP adduct per 10⁷ bases in the CHO genome induced about 1.5 *hprt* mutants for MeIQx; 2.5 *hprt* mutants for AαC, 2.8 *hprt* mutants for PhIP, and ~0.7 *hprt* mutants for 4-ABP per one million cells, at the dose concentrations of 1.5 μM (MeIQx and AαC), 1.2 μM (PhIP) and 2 μM (4-ABP) (53,69–71). The potential of dG-C8-HAA and dG-4-ABP adducts to induce mutations at the *hprt* locus appear similar; the different levels of mutation induced by these carcinogens in CHO cells were driven by the differing levels of adduct formation, which are dependent upon the levels of N-oxidation and ensuing activation of the N-hydroxylated metabolites by phase II enzymes. In our current study, the levels of 4-ABP and HAA adducts formed in human hepatocytes well exceed the threshold adduct levels that induced *hprt* mutations in CHO cells.

HAA-DNA adduct formation for MeIQx and PhIP was up to 100-fold greater in human hepatocytes than in rat hepatocytes (Figures 6 and 7), whereas the levels of 4-ABP-DNA adducts were similar in hepatocytes of both species. Human and rat hepatocytes displayed comparable levels of MROD activity: a measure of P450 1A2 and the principal enzyme involved in N-oxidation of HAAs (72). The discrepancy in MeIQx- and PhIP-DNA adduct formation between species can be explained, in part, by the different catalytic activities of human and rat P4501A2 orthologues towards these procarcinogens. The catalytic efficiency of human P450 1A2 is 11- and 19-fold superior than rat P450 1A2 in the N-oxidation of MeIQx and PhIP, respectively, whereas the catalytic efficiency of MROD is quite comparable for human and rat P450 1A2 (72). MeIQx and PhIP are extensively metabolized in human hepatocyte preparations (>75% of the 10 μM dose); however, both carcinogens are poorly metabolized in rat hepatocytes, and a majority of the dose remains unmetabolized, even after 24 h (37,38). There are also interspecies differences in regioselectivity of the P450 1A2 oxidation of PhIP and MeIQx. Rat P4501A2 catalyzes oxidation at the heterocyclic rings of PhIP and MeIQx to produce detoxication products; N-oxidation products of both HAAs account for less than 2% of the dose in rat hepatocytes (37,38). In contrast to the rat P450 1A2 orthologue, the catalysis of ring oxidation products of PhIP and

MeIQx, by human P450 1A2, is negligible (72,73), and a principal metabolic pathway for both HAAs in human hepatocytes occurs by P450 1A2-mediated N-oxidation (Figure 4) (37,38). In the case of 4-ABP, up to 23% of the dose (10 μ M) was converted into N-oxidation products in rat hepatocytes (74). The DNA binding data show that both human and rat P450 1A2 orthologues efficiently carry out the N-oxidation of 4-ABP and comparable levels of DNA adducts are formed in hepatocytes of both species (Figures 6 and 7).

The major pathways of metabolism of MeIQx and PhIP have been extensively characterized in rat and human hepatocytes (37,38,75,76), and in vivo of both species (40,77), but knowledge about the metabolism of A α C is limited. The metabolism of A α C was examined in the human liver tumor HepG2 cell line (78); however, these cells do not express P450 1A2 (79) and are devoid of UGTs (78), and thus, the A α C metabolites formed may not accurately reflect the biotransformation pathways that occur for A α C in humans. Recombinant human P450 1A2 enzyme does catalyze N-oxidation of the exocyclic amine group of A α C, as well as ring oxidation at the C3 and C6 atoms of the A α C heteronucleus (80,81). Both human and rodent uridine diphosphate-glucuronosyltransferases are involved in the detoxication of MeIQx and PhIP (40,75,77,82). There is also evidence for the formation of N-glucuronide conjugates of A α C in rodents (83). Glutathione S-transferases catalyze the detoxication of N-oxidized PhIP metabolites (84,85), but this family of enzymes has not been found to be involved in the detoxication of N-oxidized metabolites of MeIQx (84) or A α C (86). We have detected a number of metabolites of A α C in human hepatocytes, by LC-ESI/MS/MS (Supporting Information, Figure S-3 A and Figure S-3 B): A α C undergoes extensive oxidation, and acetyl, sulfate or glucuronide conjugates are formed. We plan to elucidate the pathways of A α C metabolism and the principal pathways involved in A α C-DNA adduct formation in hepatocytes, to establish human biomarkers of this HAA.

A α C undergoes N-oxidation by human P450 1A2, as well as P450s 1A1 and 2C9/2C10, which are highly expressed in the colon and respiratory tract (80). Moreover, human NAT1 and NAT2, and SULT1A1, which are expressed in liver and extrahepatic tissues, catalyze the binding of HONH-A α C to DNA (86,87). Thus, the potential for A α C to undergo metabolism and induce DNA damage in multiple organs that are in direct or indirect contact with tobacco smoke is great. Given the large amounts of A α C that arise in tobacco smoke (25,26) and the elevated levels of A α C found in urine of smokers in comparison to non-smokers (88), studies investigating the potential role of A α C in the carcinogenicity of liver and gastrointestinal cancers associated with tobacco smoking (89) are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

FUNDING SUPPORT

This work was funded by R01 CA122320 (E.E.B., D.G. and R.J.T.) and R01 CA134700 (E.E.B., Y.T., and R.J.T.) from the National Cancer Institute, and also partially supported by R01 CA80205 (M.C.Y.), R01 CA98497 (J.-M.Y.), and R01 CA144034 (J.-M.Y.) from the National Cancer Institute. We also thank Inserm, la Ligue contre le cancer, the region Bretagne and Anses (G.N. and S.L.) for financial aid and College Doctoral International of the universit Europenne de Bretagne for travel fellowship (G.N.)

ABBREVIATIONS

CHO

Chinese hamster ovary cells

EROD	ethoxyresorufin <i>O</i> -deethylation
HAA	heterocyclic aromatic amine
LC-ESI/MS/MS	liquid chromatography/electrospray ionization-tandem mass spectrometry
LC-ESI/MS/MSⁿ	liquid chromatography-electrospray ionization/multi-stage tandem mass spectrometry
LIT MS	linear quadrupole ion trap mass spectrometer
MROD	methoxyresorufin <i>O</i> -deethylation
NAT	<i>N</i> -acetyltransferase
SULT	sulfotransferase
4-ABP	4-aminobiphenyl
IQ	2-amino-3-methylimidazo[4,5- <i>f</i>]quinoline
AαC	2-amino-9 <i>H</i> -pyrido[2,3- <i>b</i>]indole
HONH-AαC	2-hydroxyamino-9 <i>H</i> -pyrido[2,3- <i>b</i>]indole
MeIQx	2-amino-3,8-dimethylimidazo[4,5- <i>f</i>]quinoxaline
HONH-MeIQx	2-hydroxyamino-3,8-dimethylimidazo[4,5- <i>f</i>]quinoxaline
IQx-8-COOH	2-amino-3-methylimidazo[4,5- <i>f</i>]quinoxaline-8-carboxylic acid;
8-CH₂OH-IQx	2-amino-8-(hydroxymethyl)-3-methylimidazo[4,5- <i>f</i>]quinoxaline
MeIQx-N²-Gl	<i>N</i> ² -(β-1-glucosiduronyl)-2-amino-3,8-dimethylimidazo[4,5- <i>f</i>]quinoxaline
HON-MeIQx-N²-Gl.	<i>N</i> ² -(β-1-glucosiduronyl)-2-(hydroxyamino)-3,8-dimethylimidazo[4,5- <i>f</i>]quinoxaline
MeIQx-N²-SO₃H	<i>N</i> ² -(3,8-dimethylimidazo[4,5- <i>f</i>]quinoxalin-2-yl-sulfamic acid; 2-amino-3-methylimidazo[4,5- <i>f</i>]quinoline (IQ)
PhIP	2-amino-1-methyl-6-phenylimidazo[4,5- <i>b</i>]pyridine
HONH-PhIP, HON-PhIP-N²-Gl	<i>N</i> ² -(β-1-glucosiduronyl)-2-(hydroxyamino)-1-methyl-6-phenylimidazo[4,5- <i>b</i>]pyridine
HON-PhIP-N3-Gl	<i>N</i> 3-(β-1-glucosiduronyl)-2-(hydroxyamino)-1-methyl-6-phenylimidazo[4,5- <i>b</i>]pyridine
PhIP-N²-Gl	<i>N</i> ² -(β-1-glucosiduronyl)-2-amino-1-methyl-6-phenylimidazo[4,5- <i>b</i>]pyridine
PhIP-N3-Gl	<i>N</i> 3-(β-1-glucosiduronyl)-2-amino-1-methyl-6-phenylimidazo[4,5- <i>b</i>]pyridine
dG-C8-4-ABP	<i>N</i> -(deoxyguanosin-8-yl)-4-ABP
dG-N²-N⁴-ABP	<i>N</i> -(deoxyguanosin- <i>N</i> ² -yl)-4-aminobiphenyl
dG-C8-IQ	<i>N</i> -(deoxyguanosin-8-yl)-IQ
dG-N²-IQ	5-(deoxyguanosin- <i>N</i> ² -yl)-IQ

dG-C8-AαC	<i>N</i> -(deoxyguanosin-8-yl)-AαC dG-C8-MeIQx, <i>N</i> -(deoxyguanosin-8-yl)-MeIQx
dG-<i>N</i>²-MeIQx	5-(deoxyguanosin- <i>N</i> ² -yl)-MeIQx
dG-C8-PhIP	<i>N</i> -(deoxyguanosin-8-yl)-PhIP

Reference List

1. Clayson DB. Specific aromatic amines as occupational bladder carcinogens. Natl Cancer Inst Monogr. 1981;15–19. [PubMed: 7341971]
2. IARC. Tobacco smoking. Vol. 38. International Agency for Research on Cancer; 1986. Monographs on the Evaluation of Carcinogenic Risks to Humans.
3. International Agency for Research on Cancer. Overall evaluation of carcinogenicity: An updating of IARC monographs 1–42. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1987; 7(suppl)
4. Stavric B, Klassen R, Miles W. Gas-liquid chromatographic-mass spectrometric determination of alpha- and beta-naphthylamines in FD&C Red No. 2 (amaranth). J Assoc Off Anal Chem. 1979; 62:1020–1026. [PubMed: 528442]
5. Davis VM, Bailey JE Jr. Chemical reduction of FD&C yellow No. 5 to determine combined benzidine. J Chromatogr. 1993; 635:160–164. [PubMed: 8491830]
6. Garrigos MC, Reche F, Marin ML, Jimenez A. Determination of aromatic amines formed from azo colorants in toy products. J Chromatogr A. 2002; 976:309–317. [PubMed: 12462623]
7. Lancaster FE, Lawrence JF. Determination of benzidine in the food colours tartrazine and sunset yellow FCF, by reduction and derivatization followed by high-performance liquid chromatography. Food Addit Contam. 1999; 16:381–390. [PubMed: 10755129]
8. Oh SW, Kang MN, Cho CW, Lee MW. Detection of carcinogenic amines from dyestuffs or dyed substrates. Dyes and Pigments. 1997; 33:119–135.
9. Cioni F, Bartolucci G, Pieraccini G, Meloni S, Moneti G. Development of a solid phase microextraction method for detection of the use of banned azo dyes in coloured textiles and leather. Rapid Commun Mass Spectrom. 1999; 13:1833–1837. [PubMed: 10482897]
10. Tokiwa H, Nakagawa R, Horikawa K. Mutagenic/carcinogenic agents in indoor pollutants; the dinitropyrenes generated by kerosene heaters and fuel gas and liquefied petroleum gas burners. Mutat Res. 1985; 157:39–47. [PubMed: 3892284]
11. Manabe S, Izumikawa S, Asakuno K, Wada O, Kanai Y. Detection of carcinogenic amino-alpha-carbolines and amino-gamma-carbolines in diesel-exhaust particles. Environ Pollut. 1991; 70:255–265. [PubMed: 15092136]
12. Manabe S, Kurihara N, Wada O, Izumikawa S, Asakuno K, Morita M. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine, in airborne particles and diesel-exhaust particles. Environ Pollut. 1993; 80:281–286. [PubMed: 15091848]
13. Chiang TA, Pei-Fen W, Ying LS, Wang LF, Ko YC. Mutagenicity and aromatic amine content of fumes from heated cooking oils produced in Taiwan. Food Chem Toxicol. 1999; 37:125–134. [PubMed: 10227736]
14. Patrianakos C, Hoffmann D. Chemical studies on tobacco smoke LXIV. On the analysis of aromatic amines in cigarette smoke. J Assoc Off Anal Chem. 1979; 3:150–154.
15. Luceri F, Pieraccini G, Moneti G, Dolara P. Primary aromatic amines from side-stream cigarette smoke are common contaminants of indoor air. Toxicol Ind Health. 1993; 9:405–413. [PubMed: 8367883]
16. Turesky RJ, Freeman JP, Holland RD, Nestorick DM, Miller DW, Ratnasinghe DL, Kadlubar FF. Identification of aminobiphenyl derivatives in commercial hair dyes. Chem Res Toxicol. 2003; 16:1162–1173. [PubMed: 12971805]
17. Akyuz M, Ata S. Determination of aromatic amines in hair dye and henna samples by ion-pair extraction and gas chromatography-mass spectrometry. J Pharm Biomed Anal. 2008; 47:68–80. [PubMed: 18280687]

18. Sugimura T, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci.* 2004; 95:290–299. [PubMed: 15072585]
19. Kadlubar, FF.; Beland, FA. Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamides. In: Harvey, RG., editor. *Polycyclic Hydrocarbons and Carcinogenesis*. American Chemical Society; Washington, D.C.: 1985. p. 332-370.
20. Kato R, Thorgeirsson SS. Metabolic activation of mutagenic heterocyclic aromatic amines from protein pyrolysates. *CRC Crit Rev Toxicol.* 1986; 16:307–348.
21. Sinha R, Rothman N, Salmon CP, Knize MG, Brown ED, Swanson CA, Rhodes D, Rossi S, Felton JS, Levander OA. Heterocyclic amine content in beef cooked by different methods to varying degrees of doneness and gravy made from meat drippings. *Food Chem Toxicol.* 1998; 36:279–287. [PubMed: 9651044]
22. Felton, JS.; Jagerstad, M.; Knize, MG.; Skog, K.; Wakabayashi, K. Contents in Foods, Beverages and Tobacco. In: Nagao, M.; Sugimura, T., editors. *Food Borne Carcinogens Heterocyclic Amines*. John Wiley & Sons Ltd; Chichester, England: 2000. p. 31-71.
23. Sugimura, T.; Nagao, N.; Kawachi, T.; Honda, M.; Yahagi, T.; Seino, Y.; Stao, S.; Matsukura, N.; Matsushima, T.; Shirai, A.; Sawamura, M.; Matsumoto, H. Mutagen-carcinogens in food, with special reference to highly mutagenic pyrolytic products in broiled foods. In: Hiatt, HH.; Watson, JD.; Winsten, JA., editors. *Origins of Human Cancer, Book C*. Cold Spring Harbor Laboratory; Cold Spring Harbor, NY: 1977. p. 1561-1577.
24. Yoshida D, Matsumoto T, Yoshimura R, Matsuzaki T. Mutagenicity of amino- α -carbolines in pyrolysis products of soybean globulin. *Biochem Biophys Res Commun.* 1978; 83:915–920. [PubMed: 361041]
25. Yoshida D, Matsumoto T. Amino- α -carbolines as mutagenic agents in cigarette smoke condensate. *Cancer Lett.* 1980; 10:141–149. [PubMed: 7006799]
26. Smith CJ, Qian X, Zha Q, Moldoveanu SC. Analysis of α - and β -carbolines in mainstream smoke of reference cigarettes by gas chromatography-mass spectrometry. *J Chromatogr A.* 2004; 1046:211–216. [PubMed: 15387190]
27. Zha Q, Qian NX, Moldoveanu SC. Analysis of polycyclic aromatic hydrocarbons in the particulate phase of cigarette smoke using a gas chromatographic-high-resolution mass spectrometric technique. *J Chromatogr Sci.* 2002; 40:403–408. [PubMed: 12201483]
28. Hecht SS. Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer.* 2003; 3:733–744. [PubMed: 14570033]
29. Manabe S, Tohyama K, Wada O, Aramaki T. Detection of a carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, in cigarette smoke condensate. *Carcinogenesis.* 1991; 12:1945–1947. [PubMed: 1934275]
30. Yamashita M, Wakabayashi K, Nagao M, Sato S, Yamaizumi Z, Takahashi M, Kinae N, Tomita I, Sugimura T. Detection of 2-amino-3-methylimidazo[4,5-f]quinoline in cigarette smoke condensate. *Jpn J Cancer Res.* 1986; 77:419–422. [PubMed: 3089973]
31. Otteneder M, Lutz WK. Correlation of DNA adduct levels with tumor incidence: carcinogenic potency of DNA adducts. *Mutat Res.* 1999; 424:237–247. [PubMed: 10064864]
32. Guengerich FP. Comparisons of catalytic selectivity of cytochrome P450 subfamily members from different species. *Chem-Biol Interact.* 1997; 106:161–182. [PubMed: 9413544]
33. Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF. Human cytochrome P-450PA (P450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc Natl Acad Sci USA.* 1989; 86:7696–7700. [PubMed: 2813353]
34. Turesky RJ, Lang NP, Butler MA, Teitel CH, Kadlubar FF. Metabolic activation of carcinogenic heterocyclic aromatic amines by human liver and colon. *Carcinogenesis.* 1991; 12:1839–1845. [PubMed: 1934265]
35. Guillouzo A, Morel F, Langouët S, Maheo K, Rissel M. Use of hepatocyte cultures for the study of hepatotoxic compounds. *J Hepatol.* 1997; 26(Suppl 2):73–80. [PubMed: 9204412]
36. Langouët S, Coles B, Morel F, Becquemont L, Beaune P, Guengerich FP, Ketterer B, Guillouzo A. Inhibition of CYP1A2 and CYP3A4 by oltipraz results in reduction of aflatoxin B1 metabolism in human hepatocytes in primary culture. *Cancer Res.* 1995; 55:5574–5579. [PubMed: 7585637]

37. Langouët S, Welti DH, Kerriguy N, Fay LB, Huynh-Ba T, Markovic J, Guengerich FP, Guillouzo A, Turesky RJ. Metabolism of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in human hepatocytes: 2-amino-3-methylimidazo[4,5-f]quinoxaline-8-carboxylic acid is a major detoxification pathway catalyzed by cytochrome P450 1A2. *Chem Res Toxicol*. 2001; 14:211–221. [PubMed: 11258970]
38. Langouët S, Paehler A, Welti DH, Kerriguy N, Guillouzo A, Turesky RJ. Differential metabolism of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in rat and human hepatocytes. *Carcinogenesis*. 2002; 23:115–122. [PubMed: 11756232]
39. Jarabek AM, Pottenger LH, Andrews LS, Casciano D, Embry MR, Kim JH, Preston RJ, Reddy MV, Schoeny R, Shuker D, Skare J, Swenberg J, Williams GM, Zeiger E. Creating context for the use of DNA adduct data in cancer risk assessment: I. Data organization. *Crit Rev Toxicol*. 2009; 39:659–678. [PubMed: 19743944]
40. Gu D, McNaughton L, LeMaster D, Lake BG, Gooderham NJ, Kadlubar FF, Turesky RJ. A comprehensive approach to the profiling of the cooked meat carcinogens 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline, 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine, and their metabolites in human urine. *Chem Res Toxicol*. 2010; 23:788–801. [PubMed: 20192249]
41. Frederiksen H, Frandsen H, Pfau W. Syntheses of DNA-adducts of two heterocyclic amines, 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeA{alpha}C) and 2-amino-9H-pyrido[2,3-b]indole (A{alpha}C) and identification of DNA-adducts in organs from rats dosed with MeA{alpha}C. *Carcinogenesis*. 2004; 25:1525–1533. [PubMed: 15059926]
42. Turesky RJ, Rossi SC, Welti DH, OLay J 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 N2 atoms of guanine. *Chem Res Toxicol*. 1992; 5:479–490. [PubMed: 1391614]
43. Paehler A, Richoz J, Soglia J, Vouros P, Turesky RJ. Analysis and quantification of DNA adducts of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in liver of rats by liquid chromatography/electrospray tandem mass spectrometry. *Chem Res Toxicol*. 2002; 15:551–561. [PubMed: 11952342]
44. Lin D, Kaderlik KR, Turesky RJ, Miller DW, Lay JO Jr, Kadlubar FF. Identification of N-(Deoxyguanosin-8-yl)-2-amino-1-methyl-6-phenylimidazo [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*. 1992; 5:691–697. [PubMed: 1446011]
45. Goodenough AK, Schut HA, Turesky RJ. Novel LC-ESI/MS/MSn method for the characterization and quantification of 2'-deoxyguanosine adducts of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by 2-D linear quadrupole ion trap mass spectrometry. *Chem Res Toxicol*. 2007; 20:263–276. [PubMed: 17305409]
46. Jones CR, Sabbioni G. Identification of DNA adducts using HPLC/MS/MS following in vitro and in vivo experiments with arylamines and nitroarenes. *Chem Res Toxicol*. 2003; 16:1251–1263. [PubMed: 14565767]
47. Burke MD, Mayer RT. Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P-450 binding of phenoxazone and a homologous series of its n-alkyl ethers (alkoxyresorufins). *Chem -Biol Interact*. 1983; 45:243–258. [PubMed: 6883573]
48. Eugster HP, Probst M, Würzler FE, Sengstag C. Caffeine, estradiol, and progesterone interact with human CYP1A1 and CYP1A2. *Drug Metab and Disp*. 1993; 21:43–49.
49. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72:248–254. [PubMed: 942051]
50. Beach AC, Gupta RC. Human biomonitoring and the 32P-postlabeling assay. *Carcinogenesis*. 1992; 13:1053–1074. [PubMed: 1638670]
51. Beland FA, Doerge DR, Churchwell MI, Poirier MC, Schoket B, Marques MM. Synthesis, characterization, and quantitation of a 4-aminobiphenyl-DNA adduct standard. *Chem Res Toxicol*. 1999; 12:68–77. [PubMed: 9894020]
52. Bessette EE, Spivack SD, Goodenough AK, Wang T, Pinto S, Kadlubar FF, Turesky RJ. Identification of Carcinogen DNA Adducts in Human Saliva by Linear Quadrupole Ion Trap/

- Multistage Tandem Mass Spectrometry. *Chem Res Toxicol*. 2010; 23:1234–1244. [PubMed: 20443584]
53. Turesky RJ, Bendaly J, Yasa I, Doll MA, Hein DW. The impact of NAT2 acetylator genotype on mutagenesis and DNA adducts from 2-amino-9H-pyrido[2,3-b]indole. *Chem Res Toxicol*. 2009; 22:726–733. [PubMed: 19243127]
54. Gu D, Raymundo MM, Kadlubar FF, Turesky RJ. Ultraperformance liquid chromatography-tandem mass spectrometry method for biomonitoring cooked meat carcinogens and their metabolites in human urine. *Anal Chem*. 2010
55. Bessette EE, Goodenough AK, Langouët S, Yasa I, Kozekov ID, Spivack SD, Turesky RJ. Screening for DNA adducts by data-dependent constant neutral loss-triple stage mass spectrometry with a linear quadrupole ion trap mass spectrometer. *Anal Chem*. 2009; 81:809–819. [PubMed: 19086795]
56. Beland FA, Kadlubar FF. Formation and persistence of arylamine DNA adducts in vivo. *Environ Health Perspect*. 1985; 62:19–30. [PubMed: 4085422]
57. Doerge DR, Churchwell MI, Marques MM, Beland FA. Quantitative analysis of 4-aminobiphenyl-C8-deoxyguanosyl DNA adducts produced in vitro and in vivo using HPLC-ES-MS. *Carcinogenesis*. 1999; 20:1055–1061. [PubMed: 10357788]
58. Turesky RJ, Vouros P. Formation and analysis of heterocyclic aromatic amine-DNA adducts in vitro and in vivo. *J Chromatogr B Analyt Technol Biomed Life Sci*. 2004; 802:155–166.
59. King RS, Casciano DA. NADH-Dependent reduction of carcinogenic N-hydroxy-arylamines by human and rat liver microsomes. *Proceedings of the American Association of Cancer Research*. 1998; 39
60. Turesky RJ, Parisod V, Huynh-Ba T, Langouët S, Guengerich FP. Regioselective differences in C(8)- and N-oxidation of 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline by human and rat liver microsomes and cytochromes P450 1A2. *Chem Res Toxicol*. 2001; 14:901–911. [PubMed: 11453738]
61. Tanaka T, Barnes WS, Williams GM, Weisburger JH. Multipotential carcinogenicity of the fried food mutagen 2-amino-3-methylimidazo[4,5-f]quinoline in rats. *Jpn J Cancer Res*. 1985; 76:570–576. [PubMed: 3928552]
62. Poirier MC, Beland FA. DNA adduct measurements and tumor incidence during chronic carcinogen exposure in animal models: Implications for DNA adduct-based human cancer risk assessment. *Chem Res Toxicol*. 1992; 5:749–755. [PubMed: 1489923]
63. Yu MC, Skipper PL, Tannenbaum SR, Chan KK, Ross RK. Arylamine exposures and bladder cancer risk. *Mutat Res*. 2002; 506–507:21–28.
64. Skipper PL, Tannenbaum SR, Ross RK, Yu MC. Nonsmoking-related arylamine exposure and bladder cancer risk. *Cancer Epidemiol Biomarkers Prev*. 2003; 12:503–507. [PubMed: 12814994]
65. Zhang XB, Felton JS, Tucker JD, Orlando C, Heddle JA. Intestinal mutagenicity of two carcinogenic food mutagens in transgenic mice: 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and amino(alpha)carboline. *Carcinogenesis*. 1996; 17:2259–2265. [PubMed: 8895498]
66. Okonogi H, Ushijima T, Shimizu H, Sugimura T, Nagao M. Induction of aberrant crypt foci in C57BL/6N mice by 2-amino-9H-pyrido[2,3-b]indole (A alphaC) and 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (MeIQx). *Cancer Lett*. 1997; 111:105–109. [PubMed: 9022134]
67. Schut HA, Snyderwine EG. DNA adducts of heterocyclic amine food mutagens: implications for mutagenesis and carcinogenesis. *Carcinogenesis*. 1999; 20:353–368. [PubMed: 10190547]
68. Sugimura T. Mutagens, carcinogens, and tumor promoters in our daily food. *Cancer*. 1982; 49:1970–1984. [PubMed: 7074522]
69. Metry KJ, Zhao S, Neale JR, Doll MA, States JC, McGregor WG, Pierce WM Jr, Hein DW. 2-amino-1-methyl-6-phenylimidazo [4,5-b] pyridine-induced DNA adducts and genotoxicity in chinese hamster ovary (CHO) cells expressing human CYP1A2 and rapid or slow acetylator N-acetyltransferase 2. *Mol Carcinog*. 2007; 46:553–563. [PubMed: 17295238]
70. Bendaly J, Zhao S, Neale JR, Metry KJ, Doll MA, States JC, Pierce WM Jr, Hein DW. 2-Amino-3,8-dimethylimidazo-[4,5-f]quinoxaline-induced DNA adduct formation and mutagenesis in DNA repair-deficient Chinese hamster ovary cells expressing human cytochrome P4501A1 and

- rapid or slow acetylator N-acetyltransferase 2. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:1503–1509. [PubMed: 17627018]
71. Bendaly J, Doll MA, Millner LM, Metry KJ, Smith NB, Pierce WM Jr, Hein DW. Differences between human slow N-acetyltransferase 2 alleles in levels of 4-aminobiphenyl-induced DNA adducts and mutations. *Mutat Res.* 2009; 671:13–19. [PubMed: 19682468]
72. Turesky RJ, Constable A, Richoz J, Varga N, Markovic J, Martin MV, Guengerich FP. Activation of heterocyclic aromatic amines by rat and human liver microsomes and by purified rat and human cytochrome P450 1A2. *Chem Res Toxicol.* 1998; 11:925–936. [PubMed: 9705755]
73. Wallin H, Mikalsen A, Guengerich FP, Ingelman-Sundberg I, Solberg KE, Rossland OJ, Alexander J. Differential rates of metabolic activation and detoxification of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by different cytochrome P450 enzymes. *Carcinogenesis.* 1990; 11:489–492. [PubMed: 2311193]
74. Orzechowski A, Schrenk D, Schut HA, Bock KW. Consequences of 3-methylcholanthrene-type induction for the metabolism of 4-aminobiphenyl in isolated rat hepatocytes. *Carcinogenesis.* 1994; 15:489–494. [PubMed: 8118934]
75. Turesky RJ, Bracco-Hammer I, Markovic J, Richli U, Kappeler A-M, Welte DH. The contribution of N-oxidation to the metabolism of the food-borne carcinogen 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline in rat hepatocytes. *Chem Res Toxicol.* 1990; 3:524–535. [PubMed: 2103323]
76. Alexander, J.; Heidenreich, B.; Reistad, R.; Holme, JA. Metabolism of the food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) in the rat and other rodents. In *Heterocyclic amines in cooked foods: Possible human carcinogens*. In: Adamson, RH.; Gustafsson, J-A.; Ito, N.; Nagao, M.; Sugimura, T.; Wakabayashi, K.; Yamazoe, Y., editors. 23rd Proceedings of the Princess Takamatsu Cancer Society. Princeton Scientific Publishing Co., Inc; New Jersey: 1995. p. 59-68.
77. Kulp KS, Knize MG, Fowler ND, Salmon CP, Felton JS. PhIP metabolites in human urine after consumption of well-cooked chicken. *J Chromatogr B Analyt Technol Biomed Life Sci.* 2004; 802:143–153.
78. Yuan ZX, Jha G, McGregor MA, King RS. Metabolites of the carcinogen 2-amino-alpha-carboline formed in male Sprague-Dawley rats in vivo and in rat hepatocyte and human HepG2 cell incubates. *Chem Res Toxicol.* 2007; 20:497–503. [PubMed: 17291013]
79. Aninat C, Piton A, Glaise D, Le CT, Langouet S, Morel F, Guguen-Guillouzo C, Guillouzo A. Expression of cytochromes P450, conjugating enzymes and nuclear receptors in human hepatoma HepaRG cells. *Drug Metab Dispos.* 2006; 34:75–83. [PubMed: 16204462]
80. Raza H, King RS, Squires RB, Guengerich FP, Miller DW, Freeman JP, Lang NP, Kadlubar FF. Metabolism of 2-amino-alpha-carboline. A food-borne heterocyclic amine mutagen and carcinogen by human and rodent liver microsomes and by human cytochrome P4501A2. *Drug Metab Dispos.* 1996; 24:395–400. [PubMed: 8801053]
81. Frederiksen H, Frandsen H. Impact of five cytochrome p450 enzymes on the metabolism of two heterocyclic aromatic amines, 2-amino-9H-pyrido[2,3-b]indole (AalphaC) and 2-amino-3-methyl-9H-pyrido[2,3-b]indole (MeAalphaC). *Pharmacol Toxicol.* 2003; 92:246–248. [PubMed: 12753413]
82. Malfatti MA, Dingley KH, Nowell-Kadlubar S, Ubick EA, Mulakken N, Nelson D, Lang NP, Felton JS, Turteltaub KW. The urinary metabolite profile of the dietary carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine is predictive of colon DNA adducts after a low-dose exposure in humans. *Cancer Res.* 2006; 66:10541–10547. [PubMed: 17079477]
83. Frederiksen H, Frandsen H. Excretion of metabolites in urine and faeces from rats dosed with the heterocyclic amine, 2-amino-9H-pyrido[2,3-b]indole (AalphaC). *Food Chem Toxicol.* 2004; 42:879–885. [PubMed: 15110096]
84. Lin D-X, Meyer DJ, Ketterer B, Lang NP, Kadlubar FF. Effects of human and rat glutathione-S-transferase on the covalent binding of the N-acetoxy derivatives of heterocyclic amine carcinogens in vitro: a possible mechanism of organ specificity in their carcinogenesis. *Cancer Res.* 1994; 54:4920–4926. [PubMed: 8069858]
85. Nelson CP, Kidd LC, Sauvageot J, Isaacs WB, De Marzo AM, Groopman JD, Nelson WG, Kensler TW. Protection against 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-b]pyridine cytotoxicity

- and DNA adduct formation in human prostate by glutathione S-transferase P1. *Cancer Res.* 2001; 61:103–109. [PubMed: 11196146]
86. King RS, Teitel CH, Kadlubar FF. In vitro bioactivation of N-hydroxy-2-amino-alpha-carboline. *Carcinogenesis.* 2000; 21:1347–1354. [PubMed: 10874013]
87. Glatt, H. Metabolic factors affecting the mutagenicity of heterocyclic amines. In: Skog, K.; Alexander, J., editors. *Acrylamide and Other Hazardous Compounds in Heat-Treated Foods*. Woodhead Publishing Ltd; Cambridge, England: 2006. p. 358-404.
88. Turesky RJ, Yuan JM, Wang R, Peterson S, Yu MC. Tobacco Smoking and Urinary Levels of 2-Amino-9H-Pyrido[2,3-b]Indole in Men of Shanghai, China. *Cancer Epidemiol Biomarkers Prev.* 2007; 16:1554–1560. [PubMed: 17684128]
89. IARC. Tobacco smoke and involuntary smoking. Vol. 83. International Agency for Research on Cancer; 2004. Monographs on the Evaluation of Carcinogenic Risks to Humans.

Figure 1. Chemical structures of 4-ABP and HAAs and their DNA adducts. The proposed structure of dG-N²-N⁴-4-ABP is tentative.

Figure 2.
The kinetics of 4-ABP and HAA biotransformation in human hepatocytes (F and G identity codes). Data are the mean $\pm$ SD of three independent measurements.

Figure 3.
Chemical structures of MeIQx and PhIP metabolites.

Figure 4.

The kinetics of MeIQx (upper panels) and PhIP metabolite (lower panels) formation in human hepatocytes (F and G identity codes). Data are the mean $\pm$ SD of three independent measurements.

Figure 5.

The LC-ESI/MS/MS³ traces of 4-ABP- and HAA-DNA adducts from (A) untreated hepatocytes and (B) hepatocytes treated with carcinogens for 8 h. The dG-*N*²-MeIQx adduct elutes at *t*_R 14.2 min and dG-*N*²-*N*⁴-ABP elutes at *t*_R 17.0 min.

Figure 6.

The kinetics of 4-ABP- and HAA-DNA adduct formation in hepatocytes from 4 donors, including donors F and G, which were used for kinetics of MeIQx and PhIP metabolite formation (Figure 2). Data are the average and SD of two independent measurements, except for donor E, where a single measurement was done. Time points are from left to right: T 3, 8, and 24 h. The ordinate is set at the maximum adduct value for each data set.

Figure 7.

The kinetics of 4-ABP- and HAA-DNA adduct formation in 3 different rat hepatocyte preparations. Data are the average and SD of two independent measurements. Time points are from left to right: T 3, 8, and 24 h. The ordinate is set at the maximum adduct value for each data set.

Table 1
4-ABP- and HAA-DNA Adduct Formation in Human Hepatocytes Following 24 h Incubation

	Adducts per 10 ⁷ Bases					pmol/min/mg protein	
Hepatocyte	dG-C8-4-ABP	dG-C8-AaC	dG-C8-PhIP	dG-C8-MeIQx	dG-C8-IQ	EROD	MROD
HH A	N.A.	N.A.	13.4 ± 1.7	3.4 ± 0.3	9.8 ± 1.1	N.A.	N.A.
HH B	150	32.5 ± 1.8	22.0	12.4 ± 0.6	10.3 ± 0.6	N.A.	N.A.
HH C	38.8 ± 4.8	44.4 ± 5.6	7.5 ± 1.6	13.9 ± 0.2	18.3 ± 0.8	N.A.	N.A.
HH D	100	109 ± 6.4	7.7 ± 1.0	12.2 ± 3.5	26.2 ± 1.9	N.A.	N.A.
HH E	136	9.1	9.1	27.2	N.A.	N.A.	N.A.
HH F	49.0 ± 1.7	72.0 ± 5.9	14.0 ± 0.6	19.0 ± 1.9	N.A.	N.D.	0.43 ± 0.20
HH G	87.9 ± 8.3	82.0 ± 15.4	30.6 ± 2.4	16.1 ± 1.0	13.5 ± 1.5	0.78 ± 0.48	0.38 ± 0.13
HH H	70.0 ± 5.8	31.4 ± 3.3	6.0 ± 1.0	5.1 ± 0.1	7.0 ± 2.2	0.18 ± 0.09	0.90 ± 0.27

N.A. = Not assayed; N.D. = Not detected

DNA adduct data are either single or average of duplicate measurements and the SD

EROD and MROD data (Mean ± SD, N = 4)