Hemoglobin Binding of Arylamines and Nitroarenes: Molecular Dosimetry and Quantitative Structure–Activity Relationships

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N-Oxidation and nitroreduction to yield N-hydroxyarylamines are metabolic steps that are crucial for the genotoxic properties of aromatic amines and nitroarenes, respectively. N-Hydroxyarylamines can form adducts with DNA, tissue proteins, and the blood proteins albumin and hemoglobin in a dose-dependent manner. The determination of hemoglobin adducts is a useful tool for biomonitoring exposed populations. We have established the hemoglobin binding index (HBI) (mmol compound/mole Hb) (mmol compound/kg body weight) of several aromatic amines and nitroarenes in female Wistar rats. Incorporating values obtained by other researchers in the same rat strain, the logarithm of hemoglobin binding (log HBI) was plotted against several physicochemical parameters and against calculated electronic descriptors of nitroarenes and arylamines. Most arylamines and nitroarenes form hydrolyzable (e.g., sulfonamides) adducts with hemoglobin in rats. The amount of hemoglobin binding decreases with the oxidizability of the arylamines, except for compounds that are substituted with halogens in ortho or meta position. For halogen-substituted arylamines, the amount of hemoglobin binding is directly proportional to the pKₐ. Hemoglobin binding of nitroarenes increases with the reducibility of the nitro group. The structure activity relationships (SAR) for hemoglobin binding of nitroarenes and arylamines are comparable. The SAR found for hemoglobin binding were compared with the SAR found in the literature for mutagenicity, carcinogenicity, and cytotoxicity of arylamines and nitroarenes. In general, the mutagenicity or carcinogenicity of arylamines increases with their oxidizability. This first set of data suggests that the levels of hemoglobin binding, mutagenicity, and carcinogenicity of arylamines are not determined by the same electronic properties of the compounds, or not by these properties alone. These results indicate that hemoglobin binding may prove not to be a useful index of the genotoxic potency of arylamines and nitroarenes. Further work will be performed to investigate whether high hemoglobin binding correlates with high cytotoxic potency of these compounds. — Environ Health Perspect 102(Suppl 6):61–67 (1994)

Key words: hemoglobin adducts, molecular dosimetry, aromatic amines, nitroarenes, QSAR

Introduction

Aromatic amines and nitroarenes are very important intermediates in industrial manufacturing of dyes, pesticides, and plastics and are significant environmental pollutants. Ring oxidation, N-glucuronidation, N-acetylation, and N-oxidation are the major metabolic pathways of arylamines in mammals [review of metabolism in (1,2)]. N-Oxidation is a crucial step in the metabolism of arylamines and aromatic amines to toxic products. Arylamines are metabolized in the liver by monoxygenases to highly reactive N-hydroxyarylamines. Nitroarenes are reduced by microorganisms in the gut or by nitroreductases and aldehyde dehydrogenase in hepatocytes to nitrosoarenes and N-hydroxyarylamines (3). N-Hydroxyarylamines can be further metabolized to N-sulfonylarylamines, N-acetoxyarylamines or N-hydroxyarylamine- N-glucuronide. These highly reactive intermediates are responsible for the genotoxic and cytotoxic effects (4–7) of this class of compounds. They react with DNA and proteins (Scheme 1). For 4-aminobiphenyl (4ABP) (8), it has been shown that the

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Scheme 1.

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**Materials and Methods**

The experimental details of the work with nitroarenes have been published elsewhere (9). The methods for the animal experiments, the isolation of hemoglobin, and the quantification of the arylamines bound to hemoglobin have been published recently (10). The aromatic amines and nitroarenes were given to female Wistar rats by gavage, and the rats were sacrificed 24 hr later. The hemoglobin was precipitated with ethanol, hydrolyzed in 0.1 M NaOH in the presence of recovery standards [e.g., 4-chloroaniline (4CA), d5-aniline], and extracted with hexane. The hexane fraction was analyzed by gas chromatography-mass spectrometry (GC-MS) with electron impact ionization in the single ion mode. Structure identification was based on the retention time and on the mass spectrum or the ratio of the main mass fragments. Arylamines with low hemoglobin binding were derivatized with pentafluoropropionic acid anhydride and analyzed by GC-MS. To establish whether the aromatic amines recovered from the alkaline hydrolysis were covalently bound, all samples were extracted with hexane at neutral pH and analyzed by GC-MS.

The electronic properties of the arylamines and the nitroarenes were calculated using the programs Modified Neglect of Differential Overlap (MND0), Austin Model 1 (AM1), and Parametric Method number 3 (PM3), which are part of MOPAC 6.0 (Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN) (11). The oxidizability of arylamines was determined experimentally by HPLC equipped with an electrochemical detector (10). The electrode potential was decreased stepwise (0.05 V) from 1 to 0.4 V. The peak integrals obtained (average of two injections) were plotted against the electrode potential. The half wave oxidation potential (E(1/2)) was obtained from the resulting hydrodynamic voltammograms. The values obtained are listed in Table 1.

**Results**

**Hemoglobin Binding of Arylamines and Nitroarenes**

Rats were dosed with arylamines or nitroarenes and sacrificed after 24 hr. Hemoglobin was hydrolyzed with NaOH. The released arylamine was extracted in hexane and analyzed by GC-MS. The results are summarized in Figures 1A, B, and C. The following structure activity relationships were found:

- The highest hemoglobin binding was obtained with compounds with a halogen in para position. A chlorine atom in ortho position reduces the formation of hemoglobin adducts drastically (1000-fold, for 2CA compared to 4CA). An additional ortho chlorine atom, as in 2,6-dichloroaniline (26DCA) or 2,3,4,5,6-pentachloroaniline (PCA), abolishes hemoglobin binding totally. All alkyl substituted amines have lower hemoglobin binding index (HBI) [(mmole compound/mole Hb)/(mmole compound/kg body weight)] than aniline. The HBI of 3-ethylaniline (3EA) is higher than that of 2EA or 4EA. This might be explained by the fact that the oxidation of alkyl groups in ortho or para position to an amino group is facilitated compared with that of alkyl groups in meta position. Two methyl groups in ortho position, as in 2,6-dimethylaniline (26DMA) or 2,4,6-trimethylaniline (246TMA), almost abolish hemoglobin binding.

In general, lower hydrolyzable hemoglobin-adduct levels were found in rats that were given nitroarenes than in rats that were dosed with an equimolar amount of the corresponding arylamines [except for nitrobenzene (NB) and 4-fluoronitrobenzene (4FBNB)]. The SAR of nitroarenes and arylamines are similar (Figure 2). Highest hemoglobin binding was found for 4-chloronitrobenzene (4CNB) or 4-bromonitrobenzene (4BrNB). The least binding
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Figure 1. (A) Hemoglobin binding of arylamines in rats. The logarithm of the hemoglobin binding index (log HBI) was plotted against the relative stability of the corresponding nitrenium ion. Except for 4-aminobiphenyl (4ABP) and 4-(trifluoromethyl)aniline (4TFA), all para- and alky1-substituted arylamines (19 compounds) have been included in the regression analysis: log HBI = 1.31-0.194 \text{AMNDOHF}, \ r = -0.92. The abbreviations for the compounds are mentioned in the text, except for 4-bromoaniline (4BA), 4-iodoaniline (4IA), 2,5-dimethylaniline (25DMA), 2,6-dimethylaniline (26DMA), 3,4-dimethylaniline (34DMA), and 3,5-dimethylaniline (35DMA). (B) Hemoglobin binding of arylamines in rats. The log HBI of aromatic amines with a halogen as a substituent was plotted against the pK_a; log HBI = -2.82 + 1.21 pK_a; r = 0.81.

Figure 2. Hemoglobin binding of arylamines and nitroarenes in rats. The logarithm of the hemoglobin binding index (log HBI) of the arylamines was plotted against the log HBI of the corresponding nitroarenes: log HBI (amines) = 0.86 + 0.65 log HBI (nitroarenes), \ r = 0.91. The abbreviations used in the figure are for the arylamines.

Figure 3. Hemoglobin binding of nitroarenes in rats. The logarithm of the hemoglobin binding index (log HBI) was plotted against the energy levels of the lowest unoccupied molecular orbital (E_LUMO) of the nitroarenes, calculated by the semiempirical method AM1. All para- and alky1-substituted nitroarenes fit onto the regression curve: log HBI = -6.26-2.26 E_LUMO, \ r = -0.85. 3-Chloro-4-fluoronitrobenzene (3C4FN) and 2,4-difluoronitrobenzene (2DFNB) are outliers. The HBI values published previously for nitrobenzene (NB) and 4-nitrobiphenyl (NB) were 79 (40) and 30 (41), respectively. The abbreviations for the compounds are mentioned in the text, except for 2-methylnitrobenzene (2MNB) and 3-methylnitrobenzene (3MNB).

Arylamines. In order to bind to hemoglobin, arylamines first have to be oxidized to \text{N}-hydroxyarylamines. In the liver this process is mostly catalyzed by cytochrome P450. The product distribution of this oxidation process is described best with a nitrenium ion as an intermediate (10,13). In several studies, nitrenium ions have been postulated to be the ultimate carcinogens derived from arylamines (14-20). The electronic properties of the arylamines and of the corresponding nitrenium ions were calculated with the semiempirical programs MNDO, AM1, and PM3. All calculations were performed with the hydrogens of the amino group coplanar to the benzene ring, as the initial geometry of the arylamines. The initial geometry for the calculations of \text{ortho}-substituted methyl compounds is critical. Interestingly, equivalent starting geometries do not always yield the most stable conformation for all three programs. The most stable structures are obtained when the dihedral angle of C1-C2-CH2-H is 60° for MNDO and AM1 calculations. However, for PM3 calculations the most stable conformation was obtained starting with a dihedral angle of 0°. The energy differences between the two conformations are up to 1.7 kcal/mole for AM1 and MNDO, and 0.5 kcal/mole for PM3 calculations. The most stable geometry obtained for 2EA, 3EA, and 4EA is with the second carbon of the ethyl group out of the benzene ring plane with a dihedral angle C1-C2-CH2-CH3 of 90°. This change increases the stability of the conformer by approximately 2 kcal/mole for MNDO calculations and up to 1.1 kcal/mole for AM1 and PM3 calculations. However, the most stable structure for the nitrenium ion of 2EA has a dihedral angle C1-C2-CH2-CH3 of 0°. Care must be taken with the initial geometry of 4-methylmercaptoaniline (4MSA) for MNDO calculations. A dihedral angle C3-C4-S-CH3 of 90° increases the stability of this amine by 1.8 kcal/mole. The stability of the two possible rotamers of unsymmetrically substituted nitrenium ions were compared. In the previous publication (10) only the \text{anti}-conformer with the single proton on the nitrogen on the less substituted side was studied; H1-N1-C1-C2 = 180°. This was found not to represent the most stable conformer in all cases. Therefore, for the present study we have calculated stability of the syn conformer, with the proton on the nitrogen on the more substituted side with a dihedral angle H1-N1-C1-C2 = 0°. We found that compounds with ethyl or methyl groups in \text{ortho} position the anti conformers are up to 0.9 kcal/mole more stable. Nitrenium ions with an \text{ortho} chloro group are more stable in the syn conformation as calculated by MNDO and AM1. However, with PM3 calculations the anti conformation is up to 3.6 kcal/mole more stable. The syn conformation is more stable for most \text{meta}-substituted compounds. The values of the most stable conformers have been summarized in Table 1. The enthalpy change $\Delta$MNDHOF of the isodesmic reaction (equation 1) yields a value for the stability of the nitrenium ions relative to that of aniline: $\Delta$MNDHOF = MNDHOF(aniline)-MNDHOF(amine) = [Hf(nitrenium ion of aniline) - Hf(amine)] - [Hf(nitrenium ion of amine) - Hf(amine)]; Hf=heat of formation calcu.
lated by MNDO. For amines in which nitrenium ions are less stable than the one of aniline, the enthalpy change is <0 kcal/mole.

The half wave oxidation potentials of arylamines correlate inversely with the stability of their nitrenium ions, calculated by MNDO, AM1, and PM3 with \( r = -0.95 \), \(-0.95 \), and \(0.77 \), respectively. Arylamines that form a stable nitrenium ion have a smaller oxidation potential than arylamines that form more unstable nitrenium ions (e.g., 246TMA compared to 4CA). The oxidizability of the arylamines is directly proportional to the stability (\( \Delta \text{MNDHOH} \)) of the corresponding nitrenium ions.

\[ \text{X-Ph-NH}^+ + \text{PhNH}_2 \rightarrow \text{X-Ph-NH}_2^+ + \text{PhNH}^+ \] [1]

The log HBI of all arylamines was plotted against \( \Delta \text{MNDHOH} \), \( \Delta \text{AM1H} \), and \( \Delta \text{PM3H} \). The best correlation (\( r = -0.92 \)) was found for hemoglobin binding of para-substituted and alkyl-substituted arylamines and the stability of their nitrenium ions calculated with MNDO (Figure 1A). Poorer calculations are found when log HBI was plotted against the stability of nitrenium ions calculated by AM1, PM3, and the half wave oxidation potential with \( r = -0.80 \), \(-0.70 \), and \(0.78 \), respectively. For AM1 and the half wave oxidation potential the correlations to log HBI increase to 0.91 and 0.86 if 4MSA is excluded. The compounds with halogens in ortho or meta position, 3-(trifluoromethyl)-aniline (3TFA), 3-cyanoaniline (3CNA), 4-(trifluoromethyl)-aniline (4TFA), and 4ABP do not fit the curve. From these SARs found in rats, it appears that the pharmacokinetics or the metabolism of 4ABP and 4TFA is different from that of the other para- or alkyl-substituted arylamines. This difference also might be the case in humans. Bryant et al. (21) determined the levels of hemoglobin adducts of several monocyclic and bicyclic arylamines in smokers and nonsmokers. The amount of hemoglobin adducts of arylamines correlates positively with the smoker status only for three compounds (4-ABP, 3-aminoanisole, and 2-naphthylamine). The levels of hemoglobin adducts of the other arylamines were not related to smoking habits, although these amines are present in large amounts in cigarette smoke.

Hemoglobin binding of halogen-substituted arylamines can be predicted from the \( pK_a \) values (Figure 1B). The \( pK_a \) values were taken from the literature (22, 23) except for the \( pK_a \) of 2,4-difluoroaniline (24DFA), 3-chloro-4-fluoroaniline (3C4FA), 2-chloro-4-methylaniline (2C4MA), 4-chloro-2-methylaniline (4C2MA), 5-chloro-2-methylaniline (5C2MA), and 6-chloro-2-methylaniline (6C2MA), which were estimated, according to Perrin et al. (24).

**Nitroarenes.** Nitroarenes have to be reduced to nitrosoarenes or to \( N \)-hydroxyarylamine to yield the same sulfanimide adducts as do arylamines. Therefore, hemoglobin binding of nitroarenes should depend on the ease of reduction of the nitro group. The energy level of the lowest unoccupied molecular orbital (\( E_{\text{LUMO}} \)) is a good parameter for predicting the reducibility of nitroarenes (25, 26). Thirteen nitroarenes were tested for hemoglobin binding. The logarithm of the hemoglobin binding index was plotted against \( E_{\text{LUMO}} \) (Figure 3). The para- and alkyl-substituted arylamines fit the regression curve very well. As in the case of arylamines, compounds with halogens in ortho or meta position are outliers. Analysis of a larger group of nitroarenes has been recently published (9).

Except for two arylamines (2,6-dichloroaniline [26DCA] and PCA) and two nitroarenes (24DCNB and PCNB), all arylamines and nitroarenes given to female Wistar rats formed hydrolyzable hemoglobin adducts. Therefore, for most compounds the potentially genotoxic intermediate—\( N \)-hydroxyarylamine—is bioavailable. Hemoglobin binding can be predicted with the electronic properties of the arylamines and nitroarenes. In a further analysis we determined if the same electronic properties are predictive for the carcinogenic, mutagenic, and cytotoxic potency of these arylamines and nitroarenes.

**Mutagenicity of Arylamines and Nitroarenes**

**Mutagenicity of Arylamines.** Are the electronic properties responsible for high HBI values the same as those responsible for high mutagenic and carcinogenic potency? For several arylamines, it has been shown that the mutagenic potency increases with the stability of the corresponding nitrenium ions (14, 15, 27). However, several compounds that are not mutagenic should be mutagenic according to their electronic properties, for example, aniline, 3CA, 2C4MA, and 4C2MA in Salmonella typhimurium TA98. Even with the inclusion of additional parameters (partition coefficients, the energy levels of the LUMO, and highest occupied molecular orbital of the arylamines) in a predictive equation, the mutagenicity of several arylamines is not predicted correctly (28).

In the present work, the data available for the mutagenic potency (28–32) of arylamines, expressed as logarithm of revertants per n mole compound (log MUT), were plotted against log HBI of the arylamines and the stability of the corresponding nitrenium ions. The mutagenic potency is directly proportional to the oxidizability of the arylamines (Figure 4) (e.g., 2,4,5-trimethylaniline [245TMA] is more mutagenic than 4CA), but inversely proportional to the amount of hemoglobin binding in rats (Figure 5). In addition, several arylamines (e.g., aniline, 3-chloroaniline [3CA], 2C4MA, and 4C2MA) that are not mutagenic, bind to hemoglobin.

**Mutagenicity of Nitrobenzenes.** For a set of about 20 nitroarenes (most of them were polyaromatic compounds) Klopmann...
et al. (25) and Maynard et al. (26) found a simple relationship between the electronic properties of nitroarenes and their mutagenicity. The mutagenicity increases with the ease of reduction of the nitroarenes. With the collection of further data, the models for predicting mutagenicity had to be modified. Additional factors describing the molecular dimensions, the degree of aromaticity, the hydrophobicity (33, 34, and literature cited therein), or the orientation of the nitro substituents (35 and literature cited therein) were included to improve the predictive value of the equations.

The logarithm of the mutagenicity (34, 36) of the nitroarenes which had been tested for homolog binding were plotted against the reducibility of the nitro group ($E_{LUMO}$). The mutagenic potency and the $E_{LUMO}$ fit on a linear regression line (Figure 6). The mutagenicity of nitroarenes increases with the reducibility of the nitro group. Except for NB all compounds tested which bind to hemoglobin are mutagenic. Conversely, 24DCNB is mutagenic but does not bind to hemoglobin. Although hemoglobin binding increases with the reducibility of the nitro group, the correlation of mutagenicity with hemoglobin binding is very poor. This may be a function of insufficient data points, thus further analyses are necessary.

**Carcinogenicity of Arylamines and Nitroarenes**

Since the late 1970s, there has been a great deal of interest to elucidate the chemical properties of aromatic amines and nitroarenes that are responsible for the genotoxicity of this class of compounds (16-18, 37). Much emphasis has been put on the structural features of the ultimate carcinogen. Although a bimolecular mechanism has been postulated in certain cases, most authors interpreted the carcinogenic potencies or the reactions with DNA with a nitrenium ion as an intermediate (16-18, 37). For a comparison of hemoglobin binding with the carcinogenicity of amines in rats, the $TD_{50}$ values for the reaction of 4-fluoronitrobenzene (4FNB) is not included in the regression analysis.

Figure 6. Mutagenicity of nitrobenzenes in Salmonella typhimurium TA100 (34, 36) (less data points were available for the strain TA98. (33). The logarithm of mutagenicity (log MUT) was plotted against the calculated energy levels of the lowest unoccupied molecular orbital ($E_{LUMO}$) of the nitroarenes: $r = -0.72$ for 4-fluoronitrobenzene (4FNB) is not included in the regression analysis.

Figure 7. Carcinogenicity of amines in rats. Log $1/TD_{50}$ (mmole) was plotted against the relative stability of the nitrenium ion ($\Delta M$): $r = 0.63$. The $TD_{50}$ values were obtained from Gold et al. (38).

Figure 8. Carcinogenicity and hemoglobin binding of amines in rats. Log $1/TD_{50}$ (mmole) was plotted against log hemoglobin binding index (log HBI): $r = -0.74$. The $TD_{50}$ values were obtained from Gold et al. (38).

The level of hemoglobin binding and mutagenicity is directly proportional to the reducibility of the nitroarenes, but hemoglobin binding and mutagenicity do not correlate. For amines, the electronic properties that are important for mutagenicity or carcinogenicity are not the same as those important for hemoglobin binding. Moreover, the correlation of carcinogenicity or mutagenicity of amines with the electronic properties of the corresponding nitrenium ions is not as good as that for hemoglobin binding. For an equation that better predicts carcinogenicity, other parameters that are important in the process of carcinogenesis may have to be included (e.g., cytotoxicity, $K_a$, and $V_{max}$ values of phase I and phase II enzymes, partition coefficient octanol-water).

Experiments to test for cytotoxic effects of metabolites of amines or nitroarenes have been performed with hepatocytes by O'Brien et al. (5) and de Silva et al. (4). For nitroarenes, it was shown that cytotoxicity is increased by electron-withdrawing groups in para position to the nitro group.

For amines, a new mechanism for cytotoxic effects of $N$-hydroxy-$2$-aminofluorene and $N$-hydroxy-$2$-aminophenanthrene has been proposed by Neumann et al. (6, 7). In vitro experiments with mitochondria showed that $N$-hydroxy-$2$-aminofluorene or $2$-nitrosofluorene...
caused cyanide-resistant oxygen consumption and calcium release. The formation of superoxide anion radical was demonstrated. However, several monocylic arylamines (nitrosobenzene, 2-nitrotoluene, 4-nitrosotoluene, N,N-dimethyl-4-nitrotoluene, and 4-nitrosophenol) did not cause any oxidative stress; some of them (nitrosobenzene and 4-nitrosotoluene) induced calcium release. Further work has to be performed to see which structural parameters determine the cytotoxic properties of arylamines and their metabolites.

REFERENCES

1. Beland FA, Kadlubar FF. Metabolic activation and DNA adducts of aromatic amines and nitroaromatic hydrocarbons. In: Chemical Carcinogenesis and Mutagenesis (Cooper CS, Gower PL, eds). Heidelberg: Springer-Verlag, 1990; 267–325.

2. Kadlubar FF, Beland F. Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamides. In: Polycyclic Hydrocarbons and Carcinogenesis (Harvey PG, ed). ACS Symposium Series No. 283. Washington DC: American Chemical Society, 1985; 341–370.

3. Rickert DE. Metabolism of nitroaromatic compounds. Drug Metab Rev 18:23-53 (1987).

4. Silva JM, Jatoe SD, O’Brien PJ. Glutathione may mediate the cytotoxicity of some nitrosoarenes. Prog Pharmacol Clin Pharmacol 8:289-298 (1991).

5. O’Brien PJ, Wong WC, Silva J, Khan S. Toxicity of nitrobenzene compounds towards isolated hepatocytes: dependence on reduction potential. Xenobiotica 20:945–955 (1990).

6. Neumann H-G, Ambs S, Hillesheim W. The biochemical basis of hepatotoxicity. In: Tissue Specific Toxicity: Biochemical Mechanisms (Dekant W, Neumann HG, eds). New York: Academic Press, 1992;139–162.

7. Neumann H-G, Ambs S, Bischo A. The role of non-genotoxic mechanisms in arylamine carcinogenesis. Environ Health Perspect 102(Suppl 6):000–000 (1994).

8. Kadlubar FF, Dooley KL, Teitel CH, Roberts DW, Benson RW, Butler MA, Bailey JR, Young JF, Skipper PW, Tannenbaum SR. Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. Cancer Res 51:4371–4377 (1991).

9. Sabbioni G. Hemoglobin binding of nitroarenes and quantitative structure activity relationship studies. Chem Res Toxicol 7:267–274 (1994).

10. Sabbioni G. Quantitative structure activity relationship of the N-oxidation of aromatic amines. Chem Biol Interact 81:91–117 (1992).

11. Stewart JJP. MOPAC: a semiempirical molecular orbital program. J Comp Aid Mol Design 4:1–105 (1990).

12. Sabbioni G, Neumann H-G. Biomonitoring of arylamines: hemoglobin adducts of urea and carbamate pesticides. Carcinogenesis 11:111–115 (1990).

13. Frederic CB, Hammons GJ, Beland FA, Yamazoe Y, Guengerich FP, Zenser TV, Ziegler DM, Kadlubar FF. N-Oxidation of primary arylamines in relation to chemical carcinogenesis. In: Biological Oxidation of Nitrogen in Organic Molecules: Chemistry, Toxicology and Pharmacology (Gorrod JW, Damani LA, eds). England: Ellis Horwood Ltd, 1985; 131-148.

14. Ford GP, Herman PS. Relative stabilities of nitroarenes and nitroarene ions derived from polycyclic aromatic amines. Relationship to mutagenicity. Chem Biol Interact 81:1–18 (1992).

15. Sabbioni G, Wild D. Quantitative structure activity relationships of mutagenic aromatic and heterocyclic amines. Carcinogenesis 13:709–713 (1992).

16. Scribner JD, Fisk SR. Reproduction of the major reactions of aromatic carcinogens with guanosine, using HMO-based poly-electronic perturbation theory. Tetrahedron Lett 4759-4762 (1978).

17. Scribner JD. Determinants of nucleic acid adduct formation. In: Carcinogenic and Mutagenic N-Substituted Aryl Compounds. National Cancer Institute Monograph 58, Bethesda, Maryland: National Cancer Institute, 1981; 173–182.

18. Ford GP, Scribner JD. MNDO molecular orbital study of nitroarenes derived from carcinogenic aromatic amines and amides. J Am Chem Soc 103:4281–4291 (1981).

19. Ford GP, Herman PS. Alkyl and acyl substituent effects on nitroarene ion stabilities: ab initio molecular orbital calculations. J Mol Struct 204:121–130 (1990).

20. Ford GP, Herman PS. Conformational preferences and energetic of N-O heterolyses in aryl nitroarene ion percutors: ab initio and semiempirical molecular orbital calculations. J Chem Soc Perkin Trans 2:607–616 (1991).

21. Bryant MS, Vineis P, Skipper PL, Tannenbaum SR. Hemoglobin adducts of aromatic amines: associations with smoking status and type of tobacco. Proc Natl Acad Sci USA 85:9788–9791 (1988).

22. Perrin DD. Dissociation Constants of Organic Bases in Aqueous Solution. London: Butterworths, 1965.

23. Robinson RA. Ionization constants of the six dichloroanilines and the six dichlorophenols in aqueous solution at 25°C. J Res Natl Bur Stand 86:159–167 (1966).

24. Perrin DD, Dempsey B, Serjeant E. pK, Prediction for Organic Acids and Bases. London: Chapman and Hall, 1981.

25. Klopman G, Tonucci DA, Holloway M, Rosenkranz HS. Relationship between polarographic reduction potential and mutagenicity of nitroarenes. Mutat Res 126:139–144 (1984).

26. Maynard AT, Pedersen LG, Posner HS, McKinney JD. An ab initio study of the relationship between nitroarene mutagenicity and electron affinity. Mol Pharmacol 29:629–636 (1986).

27. Ford GP, Griffin GR. Relative stabilities of nitroarene ions derived from heterocyclic amine food carcinogens: relationship to mutagenicity. Chem Biol Interact 81:19–33 (1992).

28. Debnath AK, Debnath G, Shusterman AJ, Hansch CA, QSAR investigation of the role of hydrophobicity in regulating mutagenicity in the Ames test: 1. Mutagenicity of aromatic and heteroaromatic amines in Salmonella typhimurium TA98 and TA100. Environ Mol Mutagen 19:37–52 (1992).

29. Kugler-Stegmeier ME. Erfassung der Genotoxizität von Anilin-Derivaten mit verschiedenen Testsystemen. PhD thesis, Eidgenössische Technische Hochschule Zurich Nr. 8467, Zurich, Switzerland: 1988.

30. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: IV. Results from the testing of 300 chemicals. Environ Mol Mutagen 11(Suppl 12):1–158 (1988).

31. Zeiger E, Anderson B, Haworth S, Lawlor T, Mortelmans K. Salmonella mutagenicity tests: V. Results from the testing of 311 chemicals. Environ Mol Mutagen 19(Suppl 12):1–141 (1992).

32. Kalopissi G. Structure-activity relationships of aromatic amines in the Ames Salmonella typhimurium assay. Mutat Res 246:65–66 (1991).

33. Debnath AK, Lopez de Compadre RL, Debnath G, Shusterman AJ, Hansch C. Structure-activity relationship of mutagenic aromatic and heteroaromatic nitro compounds. Correlation with molecular orbital energies and hydrophobicity. J Med Chem 34:786–797 (1991).

34. Debnath AK, Lopez de Compadre RL, Shusterman AJ, Hansch C. Quantitative structure-activity relationship investigation of
the role of hydrophobicity in regulating mutagenicity in the Ames test: 2. Mutagenicity of aromatic and heteroaromatic nitro compounds in *Salmonella typhimurium* TA100. Environ Mol Mutagen 19:53–70 (1992).

35. Jung H, Shaikh AU, Heflich RH, Fu PP. Nitro group orientation, reduction potential, and direct-acting mutagenicity of nitro-polycyclic aromatic hydrocarbons. Environ Mol Mutagen 17:169–180 (1991).

36. El-Bayoumi K, Lavoie EJ, Hecht SS, Fow EA, Hoffman D. The influence of methyl substitution on the mutagenicity of nitronaphthalenes and nitrobenzenes. Mutat Res 81:143–153 (1981).

37. Hartman GD, Schlegel HB. The relationship of the carcinogenic/mutagenic potential of arylamines to their singlet-triplet nitrenium ion energies. Chem Biol Interact 36:319–330 (1981).

38. Gold LS, Slone TH, Bernstein L. Summary of carcinogenic potency (TD_{50}) and positivity for 492 rodent carcinogens in the carcinogenic potency database. Environ Health Perspect 79:259–272 (1989).

39. Neumann H-G. Exposure control versus risk assessment: lessons from the study of genotoxic N-substituted arenes. In: Molecular Dosimetry of Human Cancer; Epidemiological Analytical and Social Considerations (Skipper PL, Groopman JD, eds). Boca Raton, Florida: CRC Press, 1991;27–52.

40. Albrecht W, Neumann H-G. Biomonitoring of aniline and nitrobenzene. Hemoglobin binding in rats and analysis of adducts. Arch Toxicol 57:1–5 (1985).

41. Suzuki J, Meguro SI, Morita O, Hirayama S, Suzuki S. Comparison of *in vivo* binding of aromatic nitro and amino compounds to rat hemoglobin. Biochem Pharmacol 38:3511–3519 (1989).