CHIMIA 49 (1995) Nr. 10 (Oktober)

Chimia 49 (1995) 374–380

ISSN 0009–4293

Comparison of Hemoglobin Binding, Mutagenicity, and Carcinogenicity of Arylamines and Nitroarenes

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Abstract. *N*-Oxidation and nitro-reduction which yield *N*-hydroxyarylamines are metabolic steps that are crucial for the genotoxic and toxic properties of aromatic amines and nitroarenes, respectively. *N*-Hydroxyarylamines form adducts with DNA, tissue proteins, and blood proteins. Except for nine compounds, it has been shown that after treatment of rats with nitroarenes ($n = 31$) or arylamines ($n = 36$) hydrolyzable hemoglobin adducts are formed as a result of the formation of the potentially genotoxic intermediate, *N*-hydroxyarylamine. Therefore, hemoglobin adducts are a good dosimeter of human exposure to a large array of arylamines and nitroarenes. The amount of hemoglobin binding decreases with the oxidizability of the arylamines, except for compounds which are substituted with halogens in the *ortho*- and/or *meta*-position. For halogen-substituted arylamines, the amount of hemoglobin binding is directly proportional to the pK_a. The level of hemoglobin binding and mutagenicity is directly proportional to the reducibility of the nitroarenes, but hemoglobin binding and mutagenicity do not correlate. 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 is needed to investigate the relationship between hemoglobin binding and cytotoxicity of these compounds. However, the prospect that other blood proteins might be more suitable as biomarkers for biological effects of nitroarenes and arylamines needs to be investigated.

1. Introduction

Aromatic amines and nitroarenes are important occupational and environmental pollutants. Ring oxidation, *N*-glucuronidation, *N*-acetylation, and *N*-oxidation are the major metabolic pathways of arylamines in mammals (review of metabolism [1]). *N*-Oxidation is a crucial step in the metabolism of arylamines and aromatic amides to toxic products. Arylamines are metabolized in the liver by monooxygenases to yield highly reactive *N*-hydroxyarylamines. Nitroarenes are reduced by microorganisms in the gut, or in hepatocytes to nitrosoarenes and *N*-hydroxyarylamines [2]. *N*-Hydroxyarylamines can be further metabolized to *N*-sulfonyloxy arylamines, *N*-acetoxy arylamines or *N*-hydroxy arylamine *N*-glucuronide. These highly reactive intermediates are responsible for the genotoxic and cytotoxic effects of this class of compounds [3]. These metabolites react with DNA and proteins (*Scheme*). Hemoglobin adducts can be used as *i*) a marker of exposure, *ii*) a marker of DNA-adducts, and *iii*) a marker for the presence of cytotoxic and genotoxic metabolites. We have investigated the structure-activity relationships (SAR) for the hemoglobin binding of several arylamines and nitroarenes in rats and compared the calculated SAR to the genotoxic and cytotoxic properties of the same compounds.

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2. Experimental

The methods for the animal experiments, the isolation of hemoglobin, and the quantification of the arylamines and nitroarenes bound to hemoglobin have been published recently [4][5]. The aromatic amines and nitroarenes were given to female *Wistar* rats by gavage and the rats were sacrificed 24 h later. Hemoglobin was hydrolyzed with sodium hydroxide (0.1M) in the presence of appropriate internal standards and extracted with hexane. The hexane fraction was analyzed by 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.

The electronic properties of the arylamines were calculated using the Austin Model 1 program (AM1), which is part of MOPAC 6.0 (Quantum Chemistry Program Exchange, Indiana University, Bloomington, IN) [6]. All calculations were performed using the default parameters. The key word PRECISE was used to increase the criteria for terminating all optimizations, electronic and geometric, by a factor of 100. All the calculations passed *Herbert*'s test in the *Broyden-Fletscher-Goldfarb-Shanno* algorithm for geometry optimization. The starting geometries were created with HyperChem 3 (*Autodesk Inc*, Sausalito, CA). The energy level of the lowest unoccupied molecular orbital (E_{LUMO}) of the nitroarenes were calculated with the program Parametric Method number 3 (PM3) which is part of HyperChem 3 (*Autodesk Inc*, Sausalito, CA). The algorithm for geometry optimization was from *Polak Ribière*. The termination conditions for the calculations with PM3 were 0.01 kcal/mol as convergence limit for the self-consistent field calculation and 0.1 kcal/(mol Å) for the root mean square of the energy gradient. The linear regression analyses were performed with the program Cricket Graph 1.2 (Computer-Associates).

3. Results and Discussion

3.1. *Calculation of the Electronic Properties of Arylamines and Nitroarenes*

The initial geometry of the arylamines for all calculations was set so that the hydrogens of the amino group are coplanar to the benzene ring. For a few anilines experimental values for the heat of formation (Hf) determined at 298.15 K are known [7]: Hf (aniline (A)) = 20.88, Hf (2-methylaniline (2MA)) = 13.47, Hf (3-methylaniline (3MA)) = 13.04, Hf (4-methylaniline (4MA)) = 13.21, Hf (2-ethylaniline (2EA)) = 9.19, Hf (3-ethylaniline (3EA)) = 8.46, Hf (4-ethylaniline (4EA)) = 8.12, Hf (2,4-dimethylaniline (24DMA)) = 5.68, Hf (2,5-

dimethylaniline (25DMA)) = 5.68, Hf (2,6-dimethylaniline (26DMA)) = 6.07, and Hf (3,4-dimethylaniline (34DMA)) = 6.26 kcal/mol. These values correspond the best to the values obtained with AM1. The initial geometry of *ortho*-substituted methyl compounds is critical. The most stable structures are obtained when the dihedral angle (C(1)–C(2)–CH_2–H) is 60°. The energy differences of the two conformations are up to 1.7 kcal/mol for AM1 calculations. The most stable geometry obtained for 2EA, 3EA, and 4EA was with the second carbon of the ethyl group out of the benzene ring plane with a dihedral angle (C(1)–C(2)–CH_2–CH_3) of 90°. However, the most stable structure for the nitrenium ion of 2EA has a dihedral angle (C(1)–C(2)–CH_2–CH_3) of 0°. The stability of the two possible rotamers – the *syn*-conformer, with the proton on the nitrogen on the more substituted side with a dihedral angle (H–N–C(1)–C(2)) equal to 0° and the *anti*-conformer with a dihedral angle (H–N–C(1)–C(2)) equal to 180° – of unsymmetrically substituted nitrenium ions were compared. We found that compounds with ethyl or methyl groups in the *ortho*-position the *anti*-conformers are up to 0.9 kcal/mol more stable. Nitrenium ions with an ortho chloro group are calculated to be more stable in the *syn*-conformation. The *syn*-conformation is more stable than the *anti*-conformers for the *meta* substituted compounds 3-chloroaniline (3CA), 3EA, 3MA, 3-chloro-4-fluoroaniline (3C4FA), 3,4-dichloroaniline (34DCA), 34DMA – for the ortho substituted compounds – 2-chloroaniline (2CA), 2,4-dichloroaniline (24DCA) and 2,4-difluoroaniline (24DFA). The values of the most stable structures are shown in *Table 1*.

For nitroarenes the initial geometry of the nitro group is very critical for the value of E_{LUMO}. For the QSAR studies the values obtained from the most stable structures were taken. Except for 24DFA the most stable structure for all *ortho*-substituted nitro compounds was with the nitro group at least 44° out of the plane of the benzene ring. For 2CA and 24DCA the energy difference to the coplanar structure was very small (0.2 kcal/mol). The values obtained are included in *Table 2*.

3.2. *Hemoglobin Binding of Arylamines and Nitroarenes*

For arylamines the following structure activity relationships (SAR) were found (*Figs. 1* and *2*). The highest hemoglobin binding was obtained with compounds with a halogen in the *para*-position. A chlorine atom in the *ortho*-position reduc-

Scheme

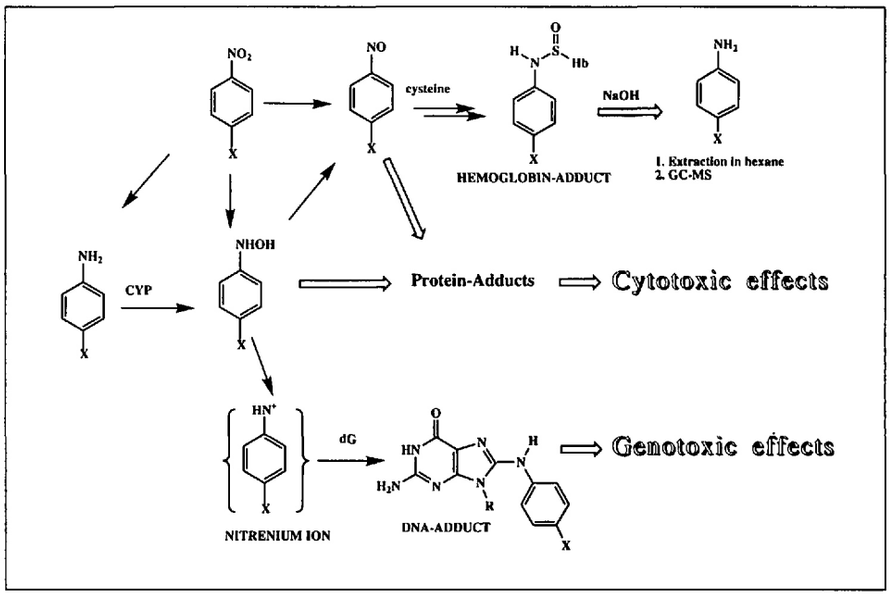

Table 1. *Heat of Formation (Hf) of Arylamines and Their Corresponding Nitrenium Ions Calculated with AM1 and Hemoglobin (Hb) Binding of Arylamines in Rats*

Arylamine[a])	HBI[b])	Hf(amine) [kcal/mol]	Hf(nitrenium) [kcal/mol]	pK_a
4MSA	3.8	19.6	224.8	4.40
A	22.0	20.5	246.9	4.58
4FA	33.0	–24.6	201.1	4.6
2CA	0.5	13.1	240.5[c])	2.65
3CA	12.5	13.3	245.0[c])	3.60
4CA	569.0	13.1	239.9	3.98
4BrA	341.0	24.6	254.8	3.86
4IA	296.0	35.9	267.3	3.78
4TFA	148.0	–137.1	105.8	2.54
2MA	4.0	13.2	234.0	4.44
3MA	4.9	13.0	237.7[c])	4.68
4MA	4.3	13.0	232.6	5.10
2EA	5.1	8.0	227.7	4.37
3EA	12.7	7.3	231.5[c])	4.70
4EA	5.8	7.1	226.2	4.35
24DMA	2.3	5.7	220.6	4.84
25DMA	7.3	5.7	224.8	4.57
26DMA	1.1	6.0	222.6	3.89
34DMA	0.7	6.1	224.5	5.22
35DMA	14.0	5.6	228.9	4.91
246TMA	0.2	–1.5	209.5	4.38
245TMA	0.7	–1.2	212.4	4.48
24DFA	32.0	–69.3	157.5	3.05
3C4FA	30.7	–29.3	201.2	3.34
24DCA	0.6	6.3	234.0[c])	2.01
26DCA	n.b.[d])	6.3	235.2	0.42
34DCA	9.0	7.5	238.7[c])	2.96
35DCA	0.6	6.7	244.0	2.38
PCA	n.b.[d])	–6.8	228.6	–
2C4MA	1.0	5.6	226.6[c])	3.04
4C2MA	28.0	5.8	227.5[c])	3.60
5C2MA	28.0	6.0	231.7[c])	3.22
6C2MA	0.8	5.9	228.3[c])	2.36
3CNA	1.5	52.1	288.0[c])	2.76
3TFA	28.4	–135.7	102.3	3.49
4ABP	344.0	45.9	259.7	4.22

[a]) Abbreviations not mentioned in the text: 4-bromoaniline (4BrA), 4-fluoroaniline (4FA), 4-iodaniline (4IA), 3,5-dimethylaniline (35DMA), 3,5-dichloroaniline (35DCA). [b]) HBI = hemoglobin binding index = [mmol compound/mol Hb]/[mmol compound/kg body weight]. [c]) *syn* conformer. [d]) n.b. = not bound, no arylamine was released after basic treatment of Hb. [e]) The most stable conformer is with the methyl group *anti* to the proton on the nitrogen.

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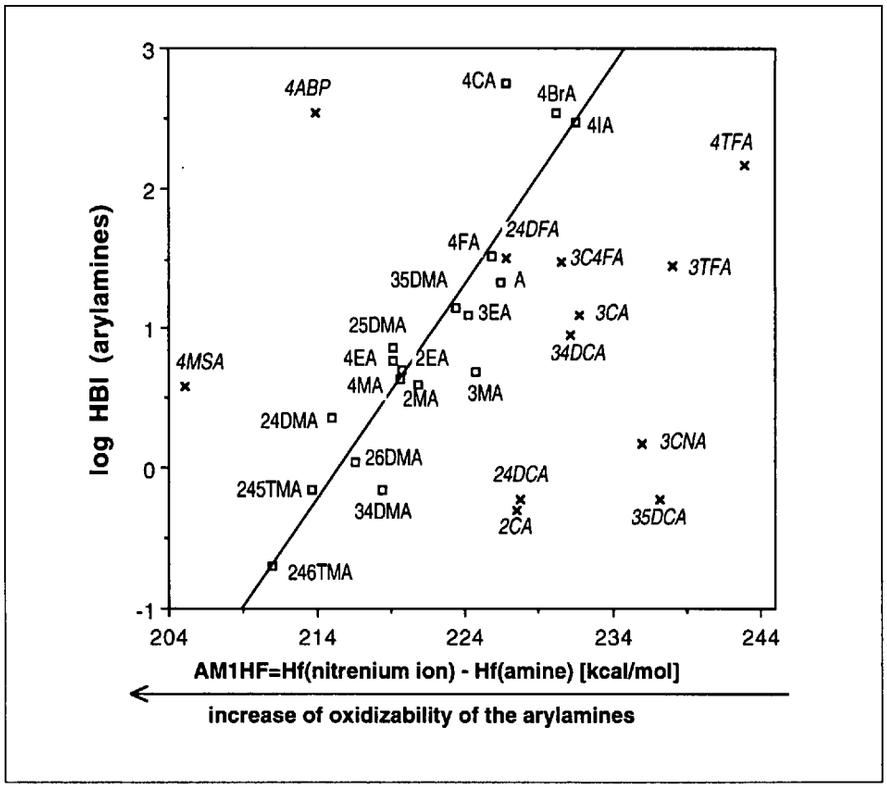

Fig. 1. *Hemoglobin binding of arylamines in rats.* The logarithm of the hemoglobin binding index (log HBI) was plotted against the relative stability of the corresponding nitrenium ion. Except for 4ABP, 4MSA, and 4TFA all *para*- and alkyl-substituted arylamines (18 compounds) have been included in the regression analysis: log HBI = –33.2 + 0.154 AM1HF, $r = 0.91$. The compounds with halogens in *ortho*- and/or *meta*-position, 3TFA, 3CNA, 4TFA, 4MSA, and 4ABP do not fit the curve. The HBI values of A, 4ABP, 4CA, 34DCA, 2MA, 3MA, 4MA, 24DMA, and 245TMA were obtained from [20].

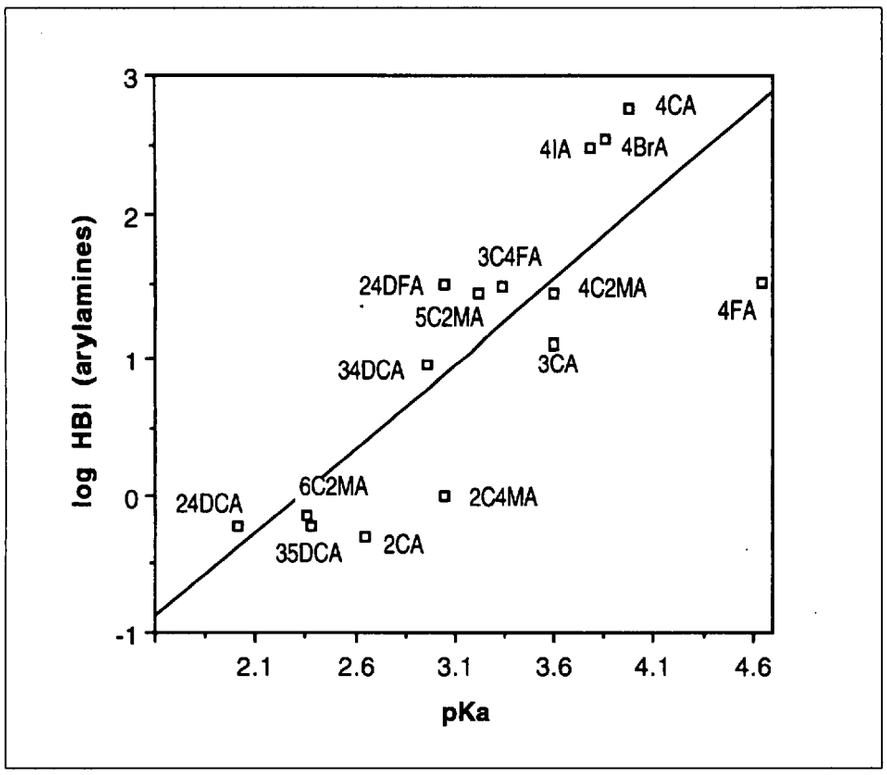

Fig. 2. *Hemoglobin binding of arylamines in rats.* The logarithm of the hemoglobin binding index (log HBI) of aromatic amines with a halogen as a substituent was plotted against the pK_a. log HBI = –2.82 + 1.21 pK_a, $r = 0.81$.

es the formation of hemoglobin adducts drastically (1000-fold for 2CA compared to 4-chloroaniline (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 indices (HBIs) than aniline (A). The HBI of 3EA is higher than that of 2EA and 4EA. This might be explained by the fact that the oxidation of alkyl groups in the *ortho*- or *para*-position to an amino group is facilitated compared with that of alkyl groups in *meta*-positions. Two methyl groups in *ortho*-positions, as in 26DMA or 2,4,6-trimethylaniline (246TMA), almost abolish hemoglobin binding.

In general, lower hydrolyzable hemoglobin adduct levels were found in rats given nitroarenes (*Fig. 3*) than in rats dosed with equimolar amounts of the corresponding arylamines (except for nitrobenzene (NB), 2-chloronitrobenzene (2CNB), 3-chloronitrobenzene (3CNB), and 4-fluoronitrobenzene (4FNB)). The SAR of nitroarenes and arylamines are similar (*Fig. 3*). Highest hemoglobin binding was found for 4-bromonitrobenzene (4BrNB) or 4-chloronitrobenzene (4CNB). The lowest binding was found with nitrobenzenes with electron donating substituents, for example 4-methylnitrobenzene (4MNB). Seven nitroarenes; 2,4-dichloronitrobenzene (24DCNB), 2,4-dimethylnitrobenzene (24DMNB), 2,6-dimethylnitrobenzene (26DMNB), 3,4-dimethylnitrobenzene (34DMNB), 2,3,4,5,6-pentachloronitrobenzene (PCNB), 2,4,6-trimethylnitrobenzene (246TMNB) and 1-nitropyrene (1NP) [8] did not form Hb-adducts which upon base treatment release the corresponding arylamine (*e.g.* 24DCA after giving rats 24DCNB).

3.3. *QSAR*

3.3.1. *Hemoglobin Binding of Arylamines and Nitroarenes*

Arylamines. In order to bind to hemoglobin, arylamines have to be oxidized to *N*-hydroxyarylamines. In the liver this process is mainly catalyzed by cytochrome P_{450}. The produet distribution of this oxidation process is described best by means of a nitrenium ion as an intermediate [9]. In several studies, nitrenium ions have been postulated to be the ultimate carcinogens derived from arylamines [10][11]. The electronic properties of the arylamines and of their corresponding nitrenium ions were calculated with the semiempirical program AM1 (*Table 1*). The difference of the Hf of the amine and the nitrenium ion yields a value for the relative stability of

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the nitrenium ions: AMI HF = Hf(nitrenium ion) – Hf(amine). The stability of the nitrenium ions correlates with the oxidizability of the arylamines (*Fig. 4*).

The logarithm of the hemoglobin binding index (log HBI) of all arylamines was plotted against AMIHF. The best correlation was found for hemoglobin binding of *para*-substituted and alkyl-substituted arylamines (*Fig. 1*). Except for 4-aminobiphenyl (4ABP), 4-methylmercaptoaniline (4MSA), and 4-(trifluoromethyl)aniline (4TFA) all *para*- and alkyl-substituted arylamines (18 compounds) have been included in the regression analysis: log HBI = –33.2 + 0.154 AMIHF, r = 0.91. The compounds with halogens in *ortho*- and/or *meta*-position, 3-(trifluoromethyl)aniline (3TFA), 3-cyanoaniline (3CNA), 4TFA, 4MSA and 4ABP do not fit on the curve. For all outliers, except for 4MSA, and 4ABP, the HBI is too low compared to the oxidizability.

Hemoglobin binding of halogen-substituted arylamines can be predicted from their pK_a values (*Fig. 2*): log HBI = –2.82 + 1.21 pK_a, r = 0.81. The pK_a values were taken [12], except for the PK_a of 24DFA, 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.* [13].

Nitroarenes. Nitroarenes have to be reduced to nitrosoarenes or to *N*-hydroxyarylamines to yield the same sulphinamide adducts as formed by arylamines. Therefore, hemoglobin binding of nitroarenes should depend on the ease of reduction of the nitro group. The E_{LUMO} is a good parameter for predicting the reducibility of nitroarenes [14]. For the present work the E_{LUMO} was calculated with PM3 (*Table 2*). The comparison with the HBI values shows that for all monocyclic nitroarenes with an E_{LUMO} higher than –0.7 except for 34DMNB or with more than one chloro group in *ortho* and *para* positions (*e.g.* 24DCNB and PCNB) do not bind to hemoglobin as sulphinamides of the parent arylamines. The log HBI was plotted against E_{LUMO} (*Fig. 5*): log HBI = –4.71–4.75 E_{LUMO}, r = –0.83. For the HBI of 1-amino-3-nitrobenzene (1A3NB) and 2-amino-4-nitrotoluene (2A4NT) only the hydrolyzable adducts resulting from the reduction of the nitro group were considered in *Table 2*. For 4-amino-2-nitrobenzene (4A2NT) only adducts with the intact nitro group were found; therefore, this compound was not included in the correlation analysis. 3-Chloro-4-fluoronitroben-

Table 2. *Heat of Formation (Hf), and E_{LUMO} of Nitroarenes Calculated with PM3, and Hemoglobin (Hb) Binding in Rats*

Nitroarene[a])	HBI[b])	E_{LUMO}	Hf(nitroarene) [kcal/mol]
NB	60.0	–1.1335	14.3
4FNB	39.5	–1.4142	–28.5
2CNB	2.1	–1.1234	11.5
3CNB	54.2	–1.3050	8.7
4CNB	215.4	–1.3565	8.2
4BrNB	225.8	–1.3906	23.2
2MNB	0.72	–0.8699	7.8
3MNB	1.0	–1.0808	4.8
4MNB	0.43	–1.0967	4.5
2ENB	0.26	–0.7626	3.7
4ENB	0.12	–1.1053	–0.1
24DMNB	n.b.[c])	–0.8532	–2.0
26DMNB	n.b.[c])	–0.6695	0.0
34DMNB	n.b.[c])	–1.0560	–3.6
35DMNB	0.63	–1.0277	–4.6
246TMNB	n.b.[c])	–0.6592	–9.6
24DFNB	2.3	–1.6595	–68.2
3C4FNB	10.0	–1.5507	–32.9
24DCNB	n.b.[c])	–1.3512	5.5
PCNB	n.b.[c])	–1.6326	–5.4
4PhNB	177.1	–1.4087	38.1
2NN	0.14[d])	–1.3863	31.4
2NF	0.10[d])	–1.3752	39.4
1NP	n.b.[c])[d])	–1.7369	58.2
13DNB	69.0[d])	–1.9551	9.0
246TNT	6.0[d])	–2.4505	3.1
24DNT	0.7[d])	–1.8197	2.3
26DNT	1.2[d])	–1.6245	6.2
1A3NB	1.2[d])[e])	–1.0601	12.3
2A4NT	1.0[d])[e])	–0.8974	8.0
4A2NT	0.1[d])[f])	–0.7099	6.2

[a]) Abbreviations not mentioned in the text: 2-methylnitrobenzene (2MNB), 3-methylnitrobenzene (3MNB), 2-ethylnitrobenzene (2ENB), 4-ethylnitrobenzene (4ENB), 3,5-dimethylnitrobenzene (35DMNB), 4-phenylnitrobenzene (4PhNB), 2-nitronaphthalene (2NN), 2-nitrofluorene (2NF), 1-nitropyrene (1NP). [b]) HBI = hemoglobin binding index = [mmol compound/mol Hb]/[mmol compound/kg body weight]. [c]) n.b. = not bound, no arylamine was released after basic treatment of Hb. [d]) These HBI were taken from [20][21]. [e]) This HBI includes only adducts resulting from nitro reduction. [f]) In this case the nitro group was not reduced, 4A2NT was released from Hb [21].

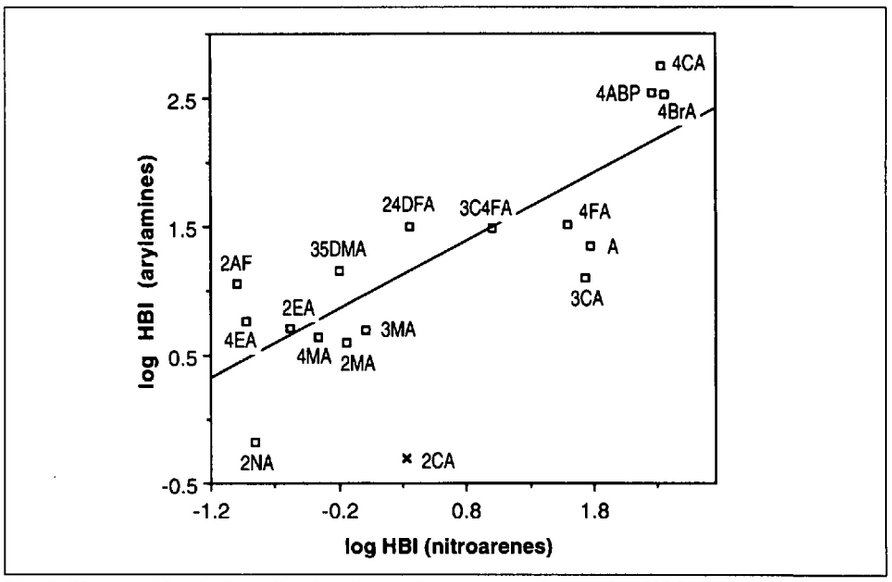

Fig. 3. *Hemoglobin binding of arylamines and nitroarenes in rats.* log HBI of the arylamines was plotted against the log HBI of the corresponding nitroarenes. log HBI (arylamines) = 0.955 + 0.531 log HBI (nitroarenes), r = 0.83. 2CA was not included in the regression analysis. The abbreviations used in the figure are for the arylamines: 2-aminofluorene (2AF), 2-naphthylamine (2NA).

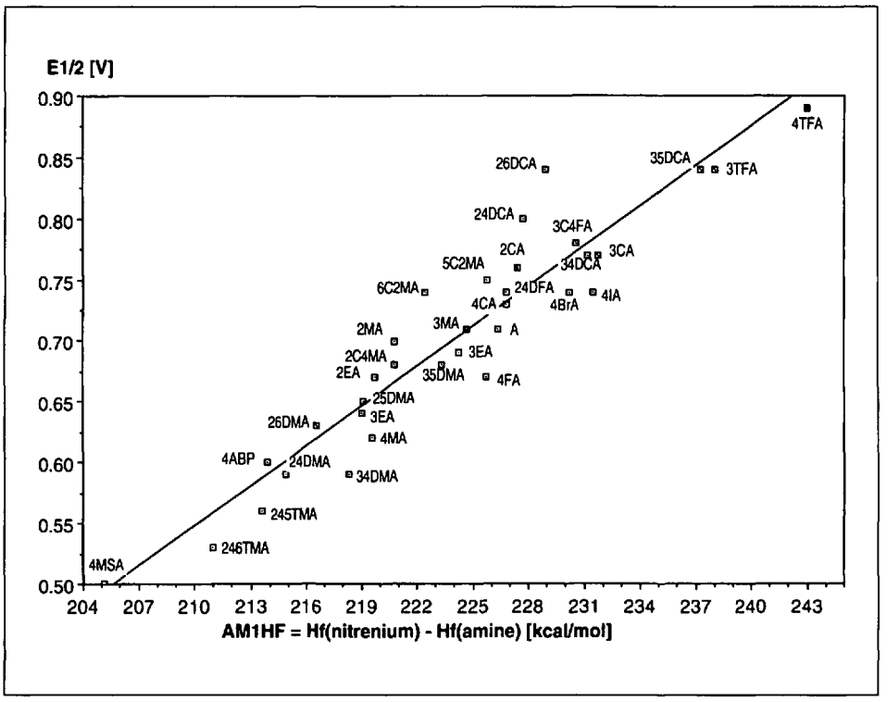

Fig. 4. *Correlation of the stability of the nitrenium ions* (AM1HF) *with the half-wave oxidation potential* ($E_{1/2}$) *determined by HPLC and an electrochemical detector* [5]; $E_{1/2} = -1.753 + 0.0952\,AM1HF$, $r = 0.95$. The electrode potential was decreased stepwise (0.05 V) from 1 to 0.4 V. The peak integrals obtained were plotted against the electrode potential. $E_{1/2}$ was obtained from the resulting hydrodynamic voltammograms.

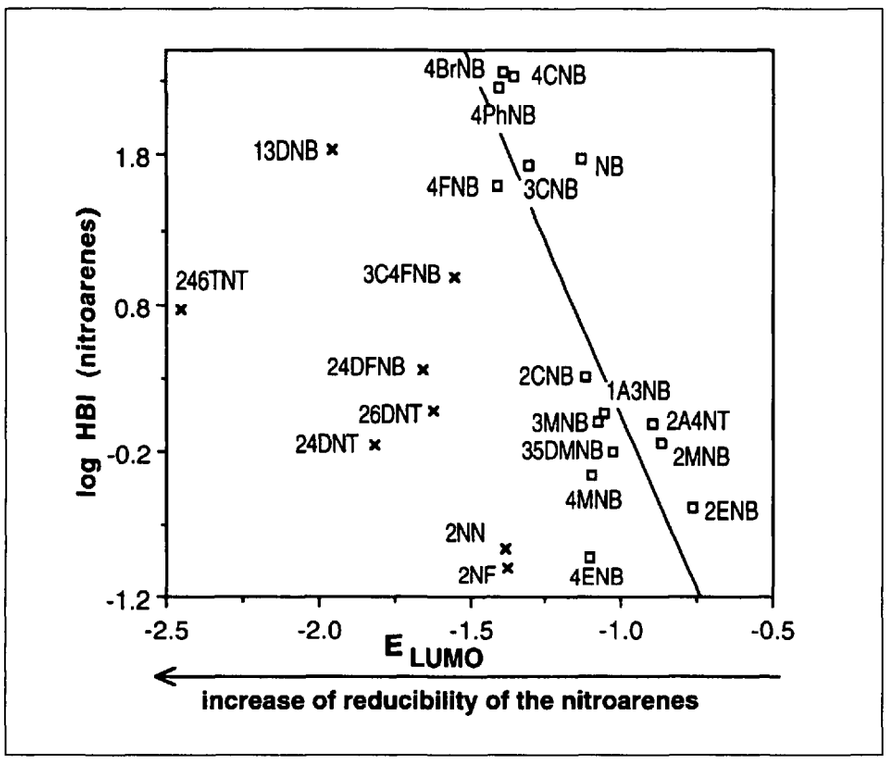

Fig. 5. *Hemoglobin binding of nitroarenes in rats.* The logarithm of the hemoglobin binding index (log HBI) was plotted against the energy levels of the lowest unoccupied molecular orbital (E_{LUMO}) of the nitroarenes calculated by the semiempirical method PM3; log HBI = –4.71 – 4.75 E_{LUMO} r = –0.83. 3C4FNB, 24DFNB, 13DNB, 24DNT, 26DNT, and 246TNT are outliers. The HBI for 1A3NB, 2A4NT, 13DNB, 24DNT, 26DNT, 2NN, and 2NF were taken from the literature [8][21].

zene (3C4FNB), 2,4-difluoronitrobenzene (24DFNB), 1,3-dinitrobenzene (13DNB), 2,4-dinitrotoluene (24DNT), 2,6-dinitrotoluene (26DNT), and 2,4,6-trinitrotoluene (246TNT) are outliers. For the outliers the HBI is much lower than predicted from their reducibility. In contrast to less reducible nitroarenes dinitroarenes and 246TNT can be activated in body compartments other than the liver and the gut [2]. Therefore, the site of activation should be different than for the monocyclic nitroarenes included in the correlation analysis. Hemoglobin binding of 24DFNB and 3C4FNB is much lower than expected from the reducibility of the nitro group. Other reaction products such as substitution of a halogen with glutathione or with hydroxy may diminish the availability of the reduced parent nitro compounds (3C4FA and 24DFA) or yield other adducts which are not detected by the current method. Interestingly, the corresponding arylamines do not fit into the QSAR found for hemoglobin binding and oxidizability of arylamines (*Fig. 1*). These initial correlations show that hemoglobin binding of nitroarenes with two nitro groups or polyaromatic compounds cannot be predicted with the equation obtained from monocyclic nitroarenes. Further descriptors such as molecular refractivity, molecular volume, and log P might have to be included to obtain predictive equations for all nitrocompounds without having to preselect the compounds according to their structure.

3.3.2. *Mutagenicity of Arylamines and Nitroarenes*

The data available for the mutagenic potency of arylamines [15], expressed as the logarithm of revertants per nmol compound (log MUT), were plotted against the 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 (*Fig. 6*) (*e.g.* 2,4,5-trimethylaniline (245TMA) is more mutagenic than

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4CA), but inversely proportional to the amount of hemoglobin binding in rats. In addition, several arylamines which are not mutagenic (*e.g.* A, 3CA, 2C4MA, and 4C2MA) bind to hemoglobin.

The log MUT [16–18] values of the nitroarenes, which have been investigated for hemoglobin binding, were plotted against the reducibility of the nitro group (E_{LUMO}). The mutagenic potency and the E_{LUMO} of mononitroarenes fit on a linear regression line (*Fig. 7*). The mutagenicity of nitroarenes increases with the reducibility of the nitro group. All compounds tested which bind to hemoglobin are mutagenic, with the exception of NB. NB binds to hemoglobin but is non-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.

3.3.3. *Carcinogenicity of Arylamines and Nitroarenes*

For a comparison of hemoglobin binding with the carcinogenicity of arylamines in rats, the TD_{50} (= daily dose, which yields tumors in 50% of the rodents) data compiled by *Gold et al.* [19] were used. TD_{50} values (mmol) of arylamines tested in rats were found for only five monocyclic arylamines. Carcinogenicity increases with the oxidizability of arylamines (*Fig. 8*). Carcinogenicity is inversely proportional to hemoglobin binding for these compounds. In addition several arylamines (*e.g.* 5C2MA, 4C2MA, 24DMA) that bind to hemoglobin in rats have been found to be carcinogenic only in mice [19]. Carcinogenicity and hemoglobin binding correlate positively only in the case of the bifunctional arylamines 3,3'-dichlorobenzidine, 4,4'-methylenedianiline, 4,4'-methylenebis(2-chloroaniline), 4,4'-oxydianiline, and benzidine (log ($1/TD_{50}$ [mmol]) = 0.76 + 0.80 log HBI; r = 0.85, data not shown). For the monocyclic nitroarenes investigated here, insufficient data are available to study the correlation of carcinogenicity with hemoglobin binding.

4. Conclusions

Most monocyclic nitroarenes and arylamines presented in this manuscript form hydrolyzable hemoglobin adducts. This demonstrates the biological availability of the potentially genotoxic and cytotoxic intermediate *N*-hydroxyarylamine

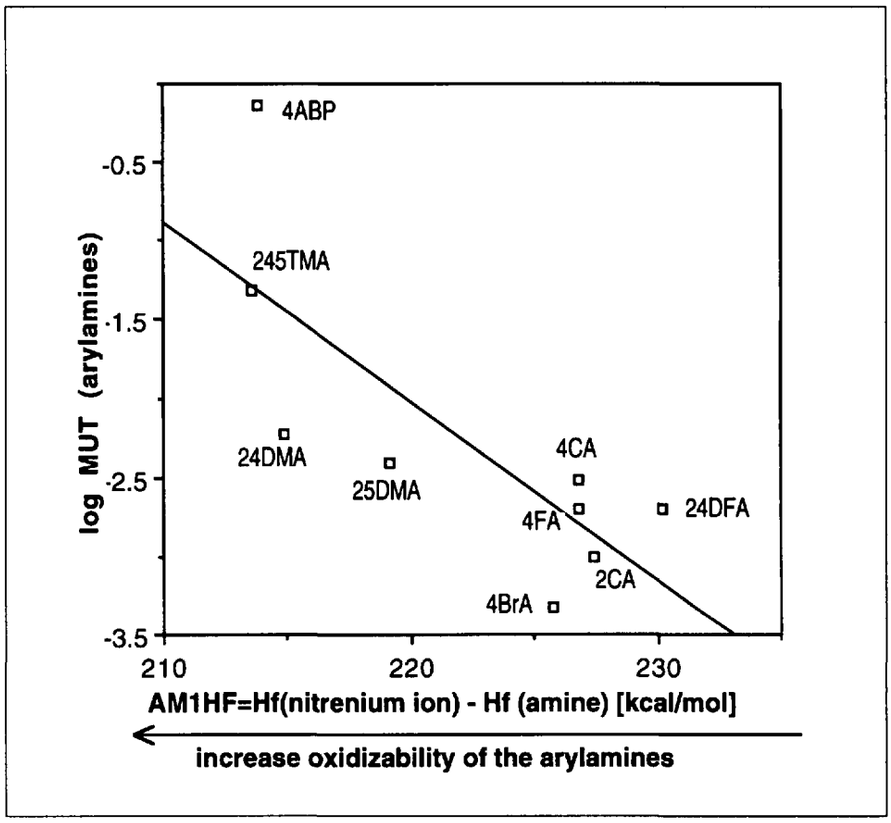

Fig. 6. *Mutagenicity of arylamines in* Salmonella typhimurium *TA98* [15]. The logarithm of mutagenicity [revertants per nmol compound] of arylamines was plotted against the relative stability of the nitrenium ions (AM1HF), log MUT = –22.9 – 0.11 AM1HF, r = –0.77.

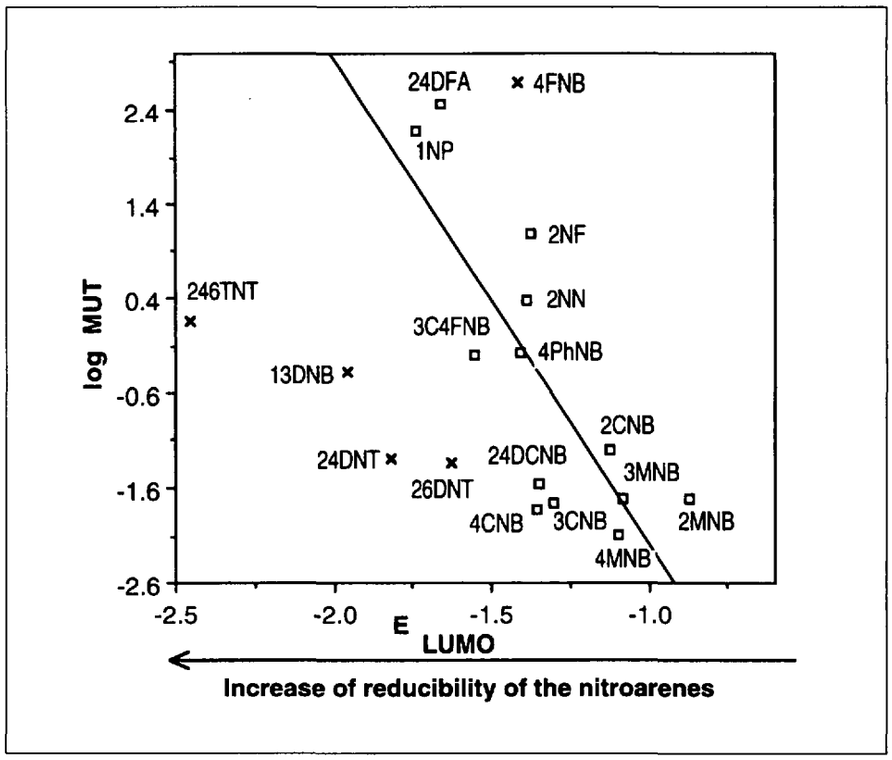

Fig. 7. *Mutagenicity of nitrobenzenes in* Salmonella typhimurium *TA100* [16][17] (fewer data points were available for the strain TA98 [16]. The logarithm of mutagenicity (log MUT) was plotted against the energy level of the lowest unoccupied molecular orbital (E_{LUMO}) of the nitroarenes; log MUT = –7.3–5.1 E_{LUMO}, r = –0.79. 4FNB is not included in the regression analysis of the mononitroarenes. Dinitro- and trinitroarenes (n = 15) fit on a different regression line [5].

(*Scheme*). The amount of hemoglobin binding decreases with the oxidizability of the arylamines, except for compounds with halogens in *ortho*- and *meta*-position. For the same compounds the mutagenicity of these arylamines increases with the oxidizability of the amino group. The few carcinogenicity studies available for monocyclic arylamines show that the carcinogenic potency of arylamines increases as well with the ease of oxidizability. Therefore, it appears that the amount of hydrolyzable hemoglobin adducts is not a good predictor for the carcinogenic and mutagenic properties of these compounds. However, it is conceivable that compounds with large hemoglobin binding are more cytotoxic. To date the cytotoxicity of arylamines and their *N*-hydroxyarylamines has been studied for only a few compounds [3]. In addition, other hemoglobin adducts which are not of the sulfinamide type and are not detected by the present method and plasma protein adducts should be investigated. These adducts may show a better correlation with biological effects. For nitroarenes the trend for all studied effects are the same, except for the carcinogenicity for which insufficient data are available.

The ease of reduction of the nitro group increases with the mutagenicity, cytotoxicity [22], and hemoglobin binding. These preliminary correlations seen for nitroarenes and arylamines should be tested with further compounds. Therefore, we strongly suggest the determination of blood protein adducts in metabolism, toxicity and carcinogenicity [23] studies of arylamines, nitroarenes and of compounds [24] which might metabolically release arylamines. Adverse health effects could then be compared with the levels of protein adducts. As blood protein adducts can be determined in humans [25], this may lead to a more reliable risk assessment. The prediction of mutagenicity and carcinogenicity of chemicals has been a topic of major interest for several research groups. The discussion of the present findings with other more general predictive models [26] goes beyond the scope of this article.

This work was supported by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* (No. 31-29387.90) and the *European Community* (STEP-CT91-0161).

Received: June 9, 1995

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