Quantum Chemical Metabolism-Based Simulation of Carcinogenic Potency of Benzene Derivatives

PAVEL N. D'YACHKOV,[1] NINA V. KHARCHEVNIKOVA,[2] ZOYA I. ZHOLDAKOVA,[2] NATHALIA FJODOROVA,[3] MARIANA NOVICH,[3] MARIAN VRACHKO[3]

[1]*Kurnakov Institute of General and Inorganic Chemistry, Russian Academy of Sciences, Moscow, Russia*

[2]*Institute of Human Ecology and Environmental Health, Russian Academy of Medical Sciences, Moscow, Russia*

[3]*National Institute of Chemistry, Ljubljana, Slovenia*

Received 26 December 2008; accepted 26 February 2009
Published online 22 July 2009 in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/qua.22226

ABSTRACT: Using an oxenoid model, we investigated dependences of carcinogenic potency of the benzenes C_6H_5-X on a nature of substituents X. According to the model, a P450 enzyme breaks a dioxygen molecule and generates the oxens, which readily react with substrates. We suggest that a stability of the intermediate OC_6H_6-X with tetrahedrally coordinated C atom relative to the molecule C_6H_5-X determines a rate of substrate biotransformation. Using MO LCAO MNDO approach, we calculated the total energies of molecules C_6H_6-X and arene oxides OC_6H_6-X. A difference ΔE_{min} of these values determines activation energy of oxidation reaction. The compounds with the low ΔE_{min} values are noncarcinogenic. Benzene derivatives with high ΔE_{min} values belong to carcinogenic compounds series. The carcinogenicity of amino- and nitro-substituted benzenes is also determined by N-oxidation of amino and reduction of the nitro group. As the phenylhydroxylamines XC_6H_4NHOH and nitrenium ions $XC_6H_4NH^+$ are the common metabolites of the nitro- and amino-substituted benzenes and nitrenium ions $XC_6H_4NH^+$ are the ultimate carcinogens, we use the differences $\Delta E_N = E(XC_6H_4NH^+) - E(XC_6H_4NHOH)$ as the second parameter characterizing the carcinogenic activity of amino- and nitro-substituted benzenes. Int J Quantum Chem 110: 1402–1411, 2010

Key words: quantum chemistry; metabolism; carcinogenicity; benzenes; oxygen; P450

Correspondence to: N. V. Kharchevnikova; e-mail: kharchevnikova_n@mail.ru

Introduction

Many chemical compounds require a biotransformation to exert the effects as the toxicants or tumor initiators [1–4]. Particularly, a concept of enzymatic activation of the procarcinogens to the proximate and ultimate carcinogens has strong basis both in in vitro and in vivo studies and in the quantum chemical calculations of structure-carcinogenic activity relationships of the polycyclic aromatic compounds [5, 6], of their alkyl-, nitro-, and amino derivatives [7–9], of the haloidalkanes and haloidalkenes [10, 11].

The oxidation catalyzed by a microsomal P450 mono-oxygenase system is a common mechanism of the foreign compounds biotransformation. Cytochrome P450 is a very large and diverse superfamily of hemoproteins.

These enzymes have been identified from all lineages of life, including mammals, birds, fish, insects, worms, sea urchins, plants, fungi, slime molds, and bacteria [12]. Human cytochromes P450 are primarily membrane-associated proteins, located either in the endoplasmic reticulum of cells or in the inner membrane of mitochondria [13–15]. The cytochromes P450 metabolize thousands of endogenous and exogenous compounds. Most cytochromes P450 can metabolize multiple substrates, and many can catalyze multiple reactions, which accounts for their central importance in metabolizing the extremely large number of endogenous and exogenous molecules. In the liver, these substrates include drugs and toxic compounds as well as metabolic products such as bilirubin (a breakdown product of hemoglobin). Cytochrome P450 enzymes are present in many other tissues of the body, including the mucosa of the gastrointestinal tract, and play important roles in hormone synthesis and breakdown (including estrogen and testosterone synthesis and metabolism), cholesterol synthesis, and vitamin D metabolism [13]. Hepatic cytochromes P450 are the most widely studied of the P450 enzymes.

The most common reaction catalyzed by cytochrome P450 is a mono-oxygenase reaction, e.g., insertion of one atom of oxygen into an organic substrate (RH), whereas the other oxygen atom is reduced to water as follows:

$$RH + O_2 + 2H^+ + 2e^- \rightarrow ROH + H_2O.$$

The benzene derivatives are known to be the typical substrates of the cytochrome P450 mediated hydroxylation. It is well-known that a rate of oxidation, a yield of the hydroxylated products, as well as a toxicity of substituted benzenes depends strongly on a nature of substituents.

In this article, we study the dependences of carcinogenicity of the benzene derivatives on the basis of quantum chemical calculations using the so-called oxenoid model [16]. According to this model, the P450 enzyme breaks the dioxygen molecule and generates the active atomic oxygen species (oxens) [17]. The oxens readily react with substrates [16, 17]. The relationships between the nature of substituents from one side and biological oxidation and acute toxicity of the benzene derivatives from another side as well as the features of metabolism of di- and trichlorinated biphenyls by bacteria were studied using an oxenoid model in our recent paper [18].

We suggest that a stability of the intermediate OC_6H_5X

with one tetrahedraly coordinated carbon atom relative to the original molecule XC_6H_5 is a factor determining the rate of enzyme-mediated substrate biotransformation.

Method of Calculations

Using MO LCAO MNDO approach, we have calculated the total energies of mono- and disubstituted benzenes XC_6H_5 and XYC_6H_4 and those of the corresponding arene oxide intermediates. A difference between these values ΔE determines approximately activation energy of oxidation reaction [16, 17]. In our calculations of benzene derivatives, we have optimized the substituent geometries only. The structure of distorted benzene ring of the intermediates OC_6H_5X was taken from paper [16]. The geometry of substituents X and Y in intermediates

OC_6H_5X and OC_6H_4XY is suggested to be the same as in the original molecules C_6H_5X and C_6H_4XY.

The carcinogenicity of amino- and nitro-substituted benzenes is also determined by N-oxidation of the amino group and by reduction of the nitro group. As the phenylhydroxylamines XC_6H_4NHOH and the nitrenium ions $XC_6H_4NH^+$ are the common metabolites of nitro- and amino-substituted benzenes and the nitrenium ions $XC_6H_4NH^+$ are the ultimate carcinogens, we have calculated a stability of the $XC_6H_4NH^+$ ions relative to the XC_6H_4NHOH molecules that is another parameter of great importance characterizing the carcinogenic activity of amino- and nitro-substituted benzenes.

Results of Calculations

In Table I, the calculated values of energies ΔE_{min} = min{ΔE(*ortho*), ΔE(*meta*), ΔE(*para*)} for the addition of oxygen to the C atoms of mono-substituted benzenes are presented together with the experimental data on the carcinogenic potency of benzene derivatives C_6H_5X. The energies ΔE(*ortho*), ΔE(*meta*), ΔE(*para*) correspond to oxidation of the *ortho*-, *meta*-, and *para*-positions, respectively. The ΔE_{min} value of the benzene C_6H_6 is taken as the energy reference point ($\Delta E(C_6H_6) = 0$). The negative ΔE_{min} values point on the stabilization of the arene oxide derivative of the C_6H_5X molecule relative to that of the benzene. The positive ΔE_{min} values points on arene oxide destabilization.

The data on carcinogenicity of compounds are taken from the Carcinogenic Potency (CP) Database—a unique and widely used international resource of the results of 6,540 chronic, long-term animal cancer tests on the 1,547 chemicals [21]. This metadata source is quality controlled and adopts statistical methods to determine carcinogenicity. In the case of the absence of data on the carcinogenicity or noncarcinogenicity of a substance in the CP database, indirect information about the carcinogenicity from a Toxnet Database [19] was used. The Toxnet is a cluster of databases on toxic effects of chemicals, including carcinogenicity and genotoxicity; particularly, it contains the CP Database and compilation of all literature data.

In the Table I, the plus signs point on carcinogenic effect for rats and/or mice, the minus signs point on the absence of carcinogenic activity.

In Table I, the calculated values ΔE_{min} vary between −0.80 and 0.20 eV. All the compounds in the Table I can be divided in two groups according to the values of the ΔE_{min} as follows:

i. Molecules with the $\Delta E_{min} < -0.17$ eV. The oxidation of carbon ring of these compounds is greatly activated relative to that of benzene and the phenols are expected to be formed readily. Almost all these compounds are noncarcinogenic, and oxidation of benzene ring seems to be the detoxication reaction here.
ii. Molecules with the $-0.17 < \Delta E_{min} < 0.20$ eV, the C_6H_6 compound itself being in this group. Here, the oxidation of compounds is only slightly activated relative to that of benzene if the $\Delta E_{min} < 0$ or even deactivated if $\Delta E_{min} > 0$. There are many carcinogenic compounds in this group. The reason is a low-rate of detoxication. Note, in a series of alkyl derivatives with X = CH_3, C_2H_5, C_3H_7, *i*-C_3H_7, and *i*-C_4H_9, only molecules with methyl and ethyl groups are carcinogenic probably because an enzymatic oxidation of a long alkyl chain is another detoxication mechanism.

In the Table II, the minimum values of ΔE, corresponding to the addition of oxygen to all nonsubstituted C atoms of benzene ring of 50 disubstituted benzene derivatives XYC_6H_4, where X and Y are Cl, NH_2, NO_2, alkyl, OCH_3, and CF_3 groups are presented. In Table II, the calculated ΔE_{min} values vary between −0.83 and 0.55 eV; that is, in a somewhat larger range than those in the Table I. Again, all the compounds can be divided in the same two groups according to the values of the ΔE_{min}. The boundary value of the parameter ΔE_{min} equal to −0.18 eV is virtually the same.

In the group of molecules with small ΔE_{min} values expected to be readily hydroxylated, there are only four carcinogens: the *ortho*- and *para*-dihydroxybenzenes and *ortho*- and *para*-metoxyphenols. The carcinogenicity of *ortho*- and *para*-dihydroxybenzenes is well-known to be due to the formation of the reactive oxygen species (ROSs) in the course of metabolism of these dihydroxybenzenes [63, 64]. According to organic chemistry data, the *ortho*- and *para*-dihydroxybenzenes HO—C_6H_4—OH are readily oxidized to the quinones O═C_6H_4═O [65]. In laboratory animals and human, this oxidation of the *ortho*- and *para*-dihydroybenzenes is catalyzed by peroxidases [63]. One-electron reduction of quinones by a NADPH-cytochrome P450 reductase leads to the formation of semiquinones O═C_6H_4═O^- anion-radicals, which rapidly react with oxygen molecule with

TABLE I

Energies ΔE_{min} for arene oxide intermediates formation and carcinogenic potency of benzene derivatives C_6H_5X.

X	ΔE_{min} (eV)	Carcinogenicity	References
NHC_2H_5	−0.80 o	−	[19, 20]
NH_2	−0.78 o	−	[19, 21]
OH	−0.68 o	−	[19, 21]
OCH_2CH_3	−0.48 p	−	[19, 22]
OCH_3	−0.39 p	−	[19, 22]
$OCOCH_3$	−0.38 p	−	[19, 23]
$NHCONHCH_3$	−0.37 p	−	[19, 24]
$NHCON(CH_3)_2$	−0.34 p	−	[19, 24]
C_6H_5	−0.17 p	−	[19, 21]
$CH{=}CH_2$	−0.17 o	+	[19, 21]
F	−0.14 o	−	[19, 25]
i-C_3H_7	−0.11 p	−	[19, 26], NTP, 1990[a]
C_3H_7	−0.08 p	−	[19, 27]
C_2H_5	−0.08 p	+	[19, 21]
CH_3	−0.07 p	+	[19, 21]
$COCH_3$	−0.07 m	−	[19, 27, 28]
i-C_4H_9	−0.06 p	−	[19, 29]
CH_2OH	−0.03 p	−	[19, 21]
H	0.00	+	[19, 21]
CHO	0.02 m	−	[19, 21]
COOH	0.04 m	−	[19, 21]
Cl	0.10 m p	+	[19, 21]
CCl_3	0.10 m	+	[19, 21]
NO_2	0.20 m	+	[19, 21]

The letters o, m, and p indicate the oxidation positions of compound.

See Ref. [20] for further details on the absence of mutagenic activity in Ames Salmonella mutagenicity tests.

See Ref. [22] for further details on negative in test on inhibition of replicative DNA synthesis in V79 Chinese hamster cells.

See Refs. [23, 24] for further details on absence of mutagenic activity in bacterial Ames tests.

See Ref. [25] for further details on not clastogenic in the micronucleus test in the mouse.

See Ref. [27] for further details on absence of mutagenic activity in bacterial Ames tests.

See Ref. [28] for further details on negative results in the tests using DNA repair-deficient bacteria. U.S. EPA classifies this chemical as D—not classifiable as to human carcinogenicity.

See Ref. [29] for further details on absence of mutagenic activity in the bacterial Ames tests.

[a] The carcinogenic potential is judged to be limited from several standpoints. The metabolic pathways of the compound do not involve any suspect reactive species. Absence of mutagenic activity both in vivo and in vitro tests, including gene mutation, chromosomal aberration, and primary DNA damage. Only a single test, a micronucleus assay, was mildly positive, and at a dose that resulted in mortality in some animals. In summary, there is not much suspicion that cumene would pose a significant carcinogenic hazard. US EPA (United States Environmental Protection Agency) has evaluated the carcinogenicity of this chemical as D - not classifiable as to human carcinogenicity.

formation of the parent quinone and superoxide anions O_2^-. The latter gives ultimately another damaging ROS such as H_2O_2, $OH^{\cdot}$, etc [64]. The *ortho*- and *para*-dihydroxybenzenes are considered to be the most hazardous metabolites of benzene itself [64]. Note, the *meta*-dihydroxybenzene is not carcinogenic, because this molecule does not form quinone [65] and is characterized by a very small value of $\Delta E_{min} = -0.80$ eV.

The carcinogenic activity of the *para*-methoxybenzene with $\Delta E_{min} = -0.37$ eV can be easily explained by formation of the carcinogenic *para*-dihydroxybenzene due to an *o*-demethylation reaction [19]. *o*-Methoxyphenol with $\Delta E_{min} = -0.52$ eV has been found to contribute to the carcinogenic effect of tobacco smoke in rats. *o*-Methoxyphenol yields carcinogenic *ortho*-dihydroxybenzene in rats [19, 49].

Up to now, we considered only one possible path of the benzene derivatives metabolism, namely that of hydroxylation. According to our results, this path leads to the decrease of carcinogenicity of the compounds under consideration. However, it is well-known [66] that the carcinogenicity of amino- and nitro-substituted benzenes is determined by N-oxidation of the amino group and reduction of the nitro group, respectively. The phenylhydroxylamines XC_6H_4NHOH are the common metabolites of nitro- and amino-substituted benzenes. The nitrenium ions $XC_6H_4NH^+$ formed in the course of Bamberger rearrangement of phenylhydroxylamines (Fig. 1) or during the heterolysis of its ethers (Fig. 2) are suggested to be the ultimate carcinogens, because these electrophilic species form adducts with DNA [66–69]. In addition to the ΔE_{min} characterizing benzene ring oxidation, we shall use the energy difference $\Delta E_N = E(XC_6H_4NH^+) - E(XC_6H_4NHOH)$ as a parameter characterizing the bioactivation of amino and nitro-substituted benzenes in the course of the metabolic transformations of amino and nitro groups. The lesser is the ΔE_N value the more stable is the nitrenium ion relative to the corresponding phenylhydroxylamine. Here, the ΔE_N value for aniline (or equally nitrobenzene) is taken as the energy reference point. In the Table III, the values of ΔE_{min} and ΔE_N parameters together with the experimental carcinogenicity data are presented. The Figure 3 shows the carcinogenicity data in the $\Delta E_N - \Delta E_{min}$ coordinates. One can see that there is a distinct separation between carcinogenic and noncarcinogenic compounds. The dotted line separates the groups of carcinogenic and noncarcinogenic compounds. Really, the noncarcinogenic compounds are

TABLE II

Energies ΔE_{min} for arene oxide intermediates formation and carcinogenic potency of benzene derivatives XYC_6H_4.

No	Substance	ΔE_{min} (eV)	Carcinogenicity	References
1.	*p*-Chloroaniline	−0.83	−	[19, 21]
2.	*o*-Aminophenol	−0.81	−	[19, 30, 31, 32]
3.	*m*-Aminophenol	−0.80	−	[19, 33, 34]
4.	Resorcinol	−0.80	−	[19, 21]
5.	*p*-Aminophenol	−0.73	−	[19, 30, 35–39]
6.	*o*-Methylphenol	−0.72	−	[19, 40, 41]
7.	*m*-Methylphenol	−0.70	−	[19, 40, 41]
8.	*m*-Chloroaniline	−0.69	−	[19, 42]
9.	*m*-Methoxyphenol	−0.69	−	[19, 43]
10.	*o*-Propylphenol	−0.66	−	[19, 44]
11.	*p*-Chlorophenol	−0.64	−	[19, 45]
12.	*o*-Aminobenzotrifluoride	−0.63	−	[19, 46]
13.	*p*-Hydroxybenzaldehyde	−0.61	−	[19, 47]
14.	*m*-Chlorophenol	−0.60	−	[19, 45]
15.	*p*-Methylphenol	−0.58	−	[19, 40, 41]
16.	*p*-Propylphenol	−0.58	−	[19, 48]
17.	*o*-Chlorophenol	−0.58	−	[19, 45]
18.	Catechol	−0.56	+	[19, 21]
19.	*m*-Aminobenzotrifluoride	−0.52	−	[19, 46]
20.	*o*-Methoxyphenol	−0.52	+	[19, 49]
21.	*o*-Nitrophenol	−0.51	−	[19, 50]
22.	*p*-Nitrophenol	−0.48	−	[19, 50]
23.	Hydroquinone	−0.47	+	[19, 21]
24.	*p*-Aminobenzotrifluoride	−0.41	−	[19, 46]
25.	*p*-Nitroaniline	−0.41	−	[19, 21]
26.	*m*-Nitrophenol	−0.39	−	[19, 50]
27.	*p*-Methoxyphenol	−0.34	+	[19, 21]
28.	*m*-Nitroanisole	−0.30	−	[19, 51]
29.	*o*, *m*, *p*-Xilene	−0.18	+	[19, 21]
30.	*o*-Ethyltoluene	−0.12	−	[19, 52, 53]
31.	*p*-Nitroanisole	−0.07	−	[19, 54], (Was not reported as carcinogen by IARC, ATSDR, NTP)
32.	*m*-Chlorotoluene	0.01	−	[19], (Was not reported as carcinogen by IARC, ATSDR, NTP)
33.	*m*-Dichlorobenzene	0.02	−	[19, 55, 56]
34.	*p*-Chlorotoluene	0.03	−	[19, 57, 58]
35.	*o*-Dichlorobenzene	0.07	−	[19, 21]
36.	*o*-Nitroanisole	0.01	+	[19, 21]
37.	*p*-Dichlorobenzene	0.10	+	[19, 21]
38.	*m*-Chlorobenzotrifluoride	0.11	−	[19, 57]
39.	*o*-Chlorotoluene	0.11	−	[19, 59]
40.	*m*-Nitrotoluene	0.13	−	[19, 60, 61]
41.	*p*-Chlorobenzotrifluoride	0.14	−	[19, 57]
42.	*o*-Nitrotoluene	0.17	+	[19, 21]
43.	*o*-Nitrosotoluene	0.21	+	[19, 21]
44.	*o*-Chlorobenzotrifluoride	0.20	−	[19, 57]

TABLE II
Continued.

No	Substance	ΔE_{min} (eV)	Carcinogenicity	References
45.	*p*-Nitrotoluene	0.24	+	[19, 21]
46.	*m*-Nitrochlorobenzene	0.26	−	[19, 61, 62]
47.	*m*-Nitrobenzotrifluoride	0.26	−	[19, 46]
48.	*o*-Nitrochlorobenzene	0.27	+	[19, 21]
49.	*p*-Nitrochlorobenzene	0.37	+	[19, 21]
50.	*p*-Nitrobenzotrifluoride	0.48	−	[19, 46]
51.	*o*-Nitrobenzotrifluoride	0.55	−	[19, 46]

See Ref. [30] for further details on *ortho*- and *para*-Aminophenols are noncarcinogenic in rats.
See Ref. [31] for further details on negative results in the Ames test on T98 strain of *S. typhimurium* with and without activation.
See Ref. [32] for further details on positive in sister chromatid exchange test in vitro.
See Ref. [33] for further details on negative in Salmonella mutagenicity tests.
See Ref. [34] for further details on negative in the mutation tests in *Neurospora crassa*.
See Ref. [35] for further details on negative results in a mutation assay involving suspension cultures and soft agar cloning.
See Ref. [36] for further details on *p*-Aminophenol has not been demonstrated to be carcinogenic.
See Ref. [37] for further details on *p*-Aminophenol produced genotoxic effects in Chinese hamster ovary and mouse lymphoma cells.
See Ref. [38] for further details on *p*-Aminophenol induced sister-chromatid exchange in vitro in human lymphocytes.
See Ref. [39] for further details on *p*-Aminophenol induced mutations in a bacterial test on *E. coli* with metabolic activation.
See Ref. [40] for further details on cresols can serve as tumor promoters for 9,10-dimethyl-1,2-benzanthracene resulting in an increased incidence of skin papillomas in mice in an initiation-promotion study.
See Ref. [41] for further details on *o*-, *m*-, *p*-cresols are not mutagenic for *Salmonella typhimurium*. A 2-year feeding study with a mixture of *m*- and *p*-cresol found no evidence of neoplastic effects in male rats. In female mice that received mean doses, the incidence of squamous cell papilloma of the forestomach was significantly increased only in the high dose group. No other significant neoplastic effect was reported in mice.
See Ref. [42] for further details on negative results in the mutation Salmonella/microsome preincubation assay.
See Ref. [43] for further details on negative results in the mutation Salmonella/microsome preincubation assay.
See Ref. [44] for further details on absence of mutagenic activity in bacterial Ames tests.
See Ref. [45] for further details on 2-Chlorophenol may have tumor promoting capability, but is not a complete carcinogen. In general, chlorophenols have been negative for mutagenicity in most prokaryotic assays and induced no increased incidence of sister chromatid exchanges in mouse testicular or bone marrow cells. The International Agency for Research on Cancer (IARC) has determined that the chlorophenols as a group, are possibly carcinogenic to man, but from these 8 chlorophenols only 2,4,6-trichlorophenol is a probable carcinogen.
See Ref. [46] for further details on absence of mutagenic activity in microbial short-term assays.
See Ref. [47] for further details on absence of mutagenic activity in bacterial Ames tests.
See Ref. [48] for further details on *p*-Propylphenol is not suspected to be a carcinogen.
See Ref. [50] for further details on neither isomer induced tumors.
See Ref. [51] for further details on absence of mutagenic activity in the bacterial Ames test and REC assay.
See Ref. [52] for further details on evidence suggesting lack of carcinogenicity of vinyl toluene in experimental animals.
See Ref. [53] for further details on *o*-Vinyl toluene has been identified as a biodegradation product of *o*-ethyltoluene.
See Ref. [54] for further details on *p*-Nitroanisole has been found to be mutagenic in the Ames test, both with and without metabolic activation, induce chromosomal aberrations in rat liver cells in vitro.
See Ref. [57] for further details on ATSDR and U.S. EPA have evaluated the carcinogenicity data for 1,3-dichlorobenzene. EPA classifies this chemical as D—not classifiable as to human carcinogenicity.
See Ref. [59] for further details on not mutagenic in Ames Salmonella/microsome assay.
See Ref. [60] for further details on absence of genotoxic effect in the tests on various strains of Salmonella and mouse lymphoma cells, in a test on chromosomal aberrations with and without metabolic activation, in an in-vivo cytogenetic test and a cell transformation test.
See Ref. [61] for further details on negative in unscheduled DNA synthesis assay in primary rat hepatocytes.
See Ref. [62] for further details on negative in chromosome aberrations and sister chromatid exchanges in Chinese Hamster ovary cells.
See Ref. [63] for further details on absence of mutagenic activity in the bacterial Ames tests.

located in the region of negative ΔE_{min} values. This region corresponds to the absence of carcinogenic activity due to a high-rate of hydroxylation. In the region of positive ΔE_{min} values, the lower values of ΔE_N correspond to carcinogenicity due to a high-rate of nitrenium ions formation. The carcinogenic com-

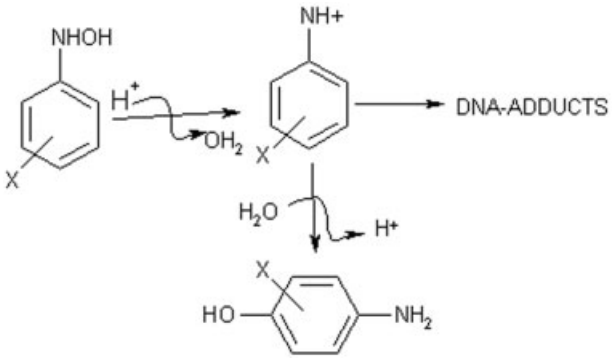

FIGURE 1. Bamberger rearrangement.

pounds are located mainly in the region of $\Delta E_{min} > 0$ and $\Delta E_N < 0$. The compounds 3 and 4, located in the region of positive ΔE_N values are carcinogenic only at high doses and only for mice. The carcinogenic effect of compound 12 shows itself also only at high doses and is observed only in one target organ in female rats.

In the terms of linear discriminant analysis [70], a canonical linear discriminant function as follows:

$$D = 0.50 - 1.35\Delta E_N + 2.80\Delta E_{min}$$

was obtained. Wilks' Lambda equals to 0.601, $P < 0.01$. Using the prior probabilities proportional to the dimension of groups, we obtained the classification functions as follows:

$$S_1 = -1.41 - 0.71\Delta E_N + 1.95\Delta E_{min}$$

$$S_2 = -0.90 + 1.584\Delta E_N - 2.80\Delta E_{min}$$

Using these functions, 84.6% of compounds were correctly classified as carcinogens or noncarcinogens.

Discussion and Conclusions

The cytochromes P450s are the principal drugs and toxicants metabolizing mono-oxygenases expressed particularly in the human liver. These enzymes contribute most extensively to the biotransformation of xenobiotics to more polar metabolites that are more readily excreted. On the other hand, cytochromes P450s are also of great importance in the bioactivation of mutagens and carcinogens, including the N-hydroxylation of arylamines and hydroxylation of aromatic compounds. The structures and the mechanisms of action of the different P450s have been well-studied experimentally. Particularly, the X-ray crystallography and spectroscopy data show that the different P450s have the active sites with somewhat different size, structure, and reactivity; however, the cytochromes P450s show overlapping substrate specificities, and individual enzymes can interact with numerous structurally diverse substrates [71–74]. This justifies our approximate approach, in which we neglect the variations of structure of different P450s families and their reactivity with different substrates, the suggested geometry of tetrahedral intermediate being consistent with the X-ray data, where the cytochrome P450-substrate complexes were detected.

In summary, taking into account our previous results [18], we conclude that the oxenoid model together with semiempirical quantum chemical calculations can reasonably explain the in vivo and in vitro experimental data on the positions of the enzyme-mediated oxidation, rate of substrate biotransformation, as well as toxicity, and carcinogenicity of the mono- and disubstituted benzene derivatives.

These simple calculations provide insight into the mechanisms of toxicity and provide criteria for separating chemicals in this class by their potential toxicity.

The further developments should include the studies of important effects of the cytochromes P450s specificities. It is really unclear if the arene oxides that are most stable ones in vacuum and even are observed in vitro [74] will be formed in vivo.

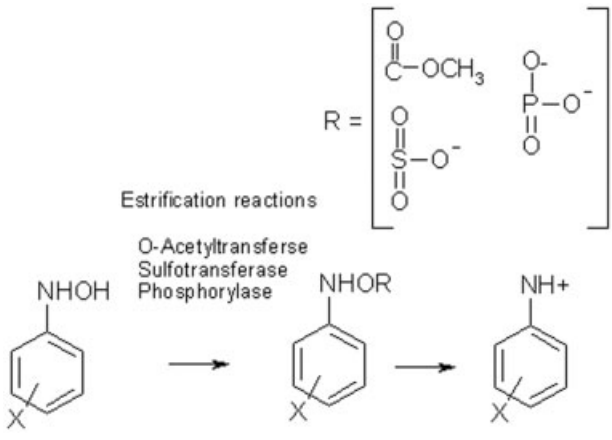

FIGURE 2. Formation of arylnitrenium ions through heterolysis of phenylhydroxylamine esters.

TABLE III

Energies ΔE_{min} for arene oxides formation, the energy differences ΔE_N of nitrenium ions and phenylhydroxylamines and carcinogenic potency of nitro and aminobenzenes.

No	Substance	ΔE_{min} (eV)	ΔE_N (eV)	Carcinogenicity	References
1.	Nitrobenzene	0.20	0	+	[19, 21]
2.	*m*-Nitrochlorobenzene	0.26	0.30	−	[19]
3.	*o*-Nitrochlorobenzene	0.27	0.20	+	[19, 21]
4.	*p*-Nitrochlorobenzene	0.37	0.22	+	[19, 21]
5.	*m*-Nitrobenzotrifluoride	0.26	0.58	−	[19]
6.	*o*-Nitrobenzotrifluoride	0.55	0.71	−	[19]
7.	*p*-Nitrobenzotrifluoride	0.48	0.81	−	[19]
8.	*o*-Nitrotoluene	0.17	−0.13	+	[19, 21]
9.	*o*-Nitrosotoluene	0.21	−0.13	+	[19, 21]
10.	*m*-Nitrotoluene	0.13	−0.03	−	[19]
11.	*p*-Nitrotoluene	0.24	−0.12	+	[19, 21]
12.	*p*-Nitrobenzoic acid	0.36	0.37	+	[19, 21]
13.	*o*-Nitroanisole	0.01	−0.59	+	[19, 21]
14.	*p*-Nitroanisole	−0.07	−0.69	−	[19]
15.	*p*-Nitroaniline	−0.41	0.91	−	[19, 21]
16.	*o*-Nitrophenol	−0.51	−0.53	−	[19]
17.	*m*-Nitrophenol	−0.39	0.15	−	[19]
18.	*p*-Nitrophenol	−0.48	−0.54	−	[19]
19.	*m*-Nitroanisole	−0.3	0.10	−	[19]
20.	Aniline	−0.78	0	−	[19, 21]
21.	*o*-Aminophenol	−0.81	−0.53	−	[19]
22.	*m*-Aminophenol	−0.80	0.15	−	[19]
23.	*p*-Aminophenol	−0.73	−0.54	−	[19]
24.	*o*-Aminobenzotrifluoride	−0.63	0.71	−	[19]
25.	*m*-Aminobenzotrifluoride	−0.52	0.58	−	[19]
26.	*p*-Aminobenzotrifluoride	−0.41	0.81	−	[19]

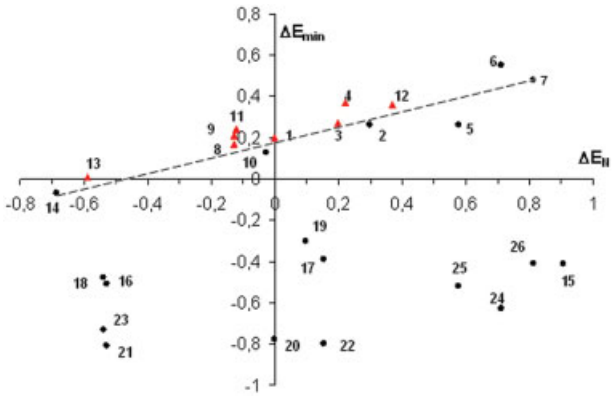

FIGURE 3. Carcinogenicity of nitro- and amino-substituted benzenes in the coordinates of parameters ΔE_N and ΔE_{min}, (eV) characterizing bioactivation and detoxication in the course of metabolism. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

ACKNOWLEDGMENT

The financial support of the European Union through CAESAR project (SSPI-022674) as well as of the Slovenian Ministry of Higher Education, Science and Technology (grant P1-017) is gratefully acknowledged.

References

1. Miller, E. C.; Miller, J. A. Pharmacol Rev 1966, 18, 805.
2. McKinney, J. D. Environ Health Perspect 1996, 104, 810.
3. Guengerich, F. P.; Shimada, T. Chem Res Toxicol 1991, 4, 391.
4. Loew, G. H.; Poulsen, M. Environ Health Perspect 1985, 61, 69.
5. Von Szenpaly, S. V. J. J Am Chem Soc 1984, 106, 6021.
6. Flesher, J. W.; Horn, J.; Lehner, A. F. J Mol Struct (THEOCHEM) 1996, 362, 29.
7. Rabinowitz, J.; Little, S.; Brown, K. Int J Quantum Chem 2002, 88, 99.

8. Hatch, F. T.; Colvin, M. E.; Seidl, E. T. Environ Mol Mutagen 1996, 27, 314.
9. Benigni, R.; Passerini, L. Mutat Res 2002, 511, 191.
10. Lag, M.; Omichinski, J. G.; Dybing, E. Chem Biol Interact 1994, 93, 73.
11. Yin, H.; Anders, M.; Korsekwa, K.; Higgins, L. A.; Thummel, K. E. Proc Natl Acad Sci USA 1995, 92, 11076.
12. Danielson, P. Curr Drug Metab 2002, 3, 561
13. Ortiz de Montellano, P. R., Ed. Cytochrome P450: Structure, Mechanism, and Biochemistry, 2nd ed.; Plenum: New York, 1995.
14. Guengerich, F. P. Chem Res Toxicol 2008, 21, 70.
15. Meunier, B.; de Visser, S. P.; Shaik, S. Chem Rev 2004, 104, 3947.
16. Korsekwa, K.; Trager, W.; Gouterman, M.; Spangler, D.; Loew, G. H. J Am Chem Soc 1985, 107, 4273.
17. Pudzianowski, A. T.; Loew, G. H. Int J Quantum Chem 1983, 23, 1257.
18. D'yachkov, P. N.; Kharchevnikova, N. V.; Dmitriev, A. V.; Kuznetsov, A. V.; Poroikov, V. V. Int J Quantum Chem 2007, 107, 2454.
19. Database Toxnet. Available at: http://toxnet.nlm.nih.gov.
20. Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K. Environ Mol Mutagen 1988, 11 (Suppl 12), 1.
21. The Carcinogenic Potency Database (CPDB). Available at: http://potency.berkeley.edu/.
22. Richard, A. M.; Hongslo, J. K.; Boone, P. F.; Holme J. A. Chem Res Toxicol 1991, 4, 151.
23. Blahova, M.; Sokolik, J.; Lahitova, N.; Murin, A. Ceska Slov Farm 1994, 43, 240.
24. Szarapinska-Kwaszewska, J.; Sobis, M.; Dudkiewicz, B.; Nawrot, E.; Rozalska, M.; Bakuniak, E.; Mikucki, J. Genet Pol 1988, 29, 227.
25. Toxikologische Bewertung. Heidelberg, Berufsgenossenschaft der chemischen Industrie 1995, 126, 13.
26. Gee, P.; Sommers, C. H.; Melick, A. S.; Gidrol, X. M.; Todd, M. D.; Burris, R. B.; Nelson, M. E.; Klemm, R. C.; Zeiger, E. Mutat Res 1998, 412, 115.
27. Japan Chemical Industry Ecology—Toxicology and Information Center, Japan; Mutagenicity Test Data of Existing Chemical Substances Based on the Toxicity Investigation of the Industrial Safety and Health Law (Suppl 2), 2000.
28. Leifer, Z.; Kada, T.; Mandel, M.; Zeiger, E.; Stafford, R.; Rosenkranz, H. S. Mutat Res 1981, 87, 211.
29. Oldham. J. W. J Appl Toxicol 1986, 6, 237.
30. Miller, J. A.; Miller E. C. Exp Med 1948, 87, 139.
31. Watanabe, T.; Kusumoto, M.; Ishihara, M.; Okumura, H.; Takase, M.; Wakisaka, H.; Hirayama, T. Eisei Kagaku 1991, 37, 512.
32. Tucker, J. D.; Auletta, A.; Cimino, M. C.; Dearfield, K. L.; Jacobson-Kram, D.; Tice, R. R.; Carrano, A. V. Mutat Res 1993, 297, 101.
33. Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K. Environ Mol Mutagen 1988, 11 (Suppl 12), 1.
34. Brockman, H. E.; de Serres, F. J.; Ong, T. M.; DeMarini, D. M.; Katz, A. J.; Griffiths, A. J.; Stafford, R. S. Mutat Res 1984, 133, 87.
35. Oberly, T. J.; Rexroat, M. A.; Bewsey, B. J.; Richardson, K. K.; Michaelis, K. C. Environ Mol Mutagen 1990, 16, 260.
36. Clayton, G. D.; Clayton, F. E., Eds. Patty's Industrial Hygiene and Toxicology, Vols. 2–F: Toxicology, 4th ed.; Wiley: New York, 1993–1994; p 973.
37. Majeska, J. B.; Holden, H. E. Environ Mol Mutagen 1995, 26, 163.
38. Takehisa, S.; Kanaya, N. Mutat Res 1982, 101, 165.
39. Japan Chemical Industry Ecology—Toxicology and Information Center, Japan; Mutagenicity Test Data of Existing Chemical Substances Based on the Toxicity Investigation of the Industrial Safety and Health Law (Suppl 2), 2000.
40. Boutwell, R. K.; Bosch, D. K. Cancer Res 1959, 19,413.
41. ATSDR (US Agency for Toxic Substances and Disease Registry) Toxicology Profile. Cresols, 2008. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp34.pdf.
42. Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W. Environ Mutagen 1987, 9, 1.
43. Florin, I.; Rutberg, L.; Curvall, M.; Enzell, C. R.; Toxicology 1980, 15, 219.
44. Arnarp, J.; Broman, S.; Dahlin, B. M.; Dahlman, O.; Enzell, C. R.; Pettersson, T. Acta Chem Scand 1991, 45, 529.
45. ATSDR Toxicological Profile for Chlorophenols. 1999. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp107.html.
46. Mazza, G.; Dacarro, C.; Bonferoni, C.; Bonferoni, B. Farmaco Ed Pratt 1986, 41, 215.
47. Japan Chemical Industry Ecology—Toxicology and Information Center, Japan; Mutagenicity Test Data of Existing Chemical Substances Based on the Toxicity Investigation of the Industrial Safety and Health Law, 1996.
48. Toxic Substances—Focus on Children Developing a Canadian List of Substances of Concern to Children's Health. Available at: http://www.pollutionprobe.org/Reports/childrentoxiclist.pdf.
49. National Research Council. Drinking Water and Health. National Academy Press: Washington, DC, 1977; Vol. 1, p 741.
50. ATSDR Toxicological Profile for Nitrophenols: 2-Nitrophenol, 4-Nitrophenol. 1992. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp50.html.
51. Shimizu, M.; Yano, E. Mutat Res 1986, 170, 11.
52. IARC. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man; World Health Organization/International Agency for Research on Cancer: Geneva, 1994; Vol. 384, p 60.
53. Kappelar, T.; Wuhrmann, K. Water Res 1978, 12, 335.
54. Ministry of Labour, Japan; Mutagenicity Test Data of Existing Chemical Substances Based on the Toxicity Investigation of the Industrial Safety and Health Law (Suppl), 1997.
55. Haworth, S.; Lawlor, T.; Mortelmans, K.; Speck, W.; Zeiger, E. Environ Mol Mutagen 1983, 5, (Suppl 1), 3.
56. ATSDR Toxicological Profile. Dichlorobenzenes. Available at: http://www.atsdr.cdc.gov/toxprofiles/tp10.html.
57. Zeiger, E.; Anderson, B.; Haworth, S.; Lawlor, T.; Mortelmans, K. Environ Mol Mutagen 1992, 19 (Suppl 21), 2.
58. Simmon, V. F. Dev Toxicol Environ Sci 1977, 2, 249.
59. National Cancer Institute. Short-Term Test Program Sponsored by the Division of Cancer Biology; National Cancer Institute, Bethesda, MD, 1997.

60. Parton, J. W.; Yount, D. J.; Garriott, M. L. Environ Mol Mutagen 1995, 26, 147.
61. Galloway, S. M.; Armstrong, M. J.; Reuben, C.; Colman, S.; Brown, B.; Cannon, C.; Bloom, A. D; Nakamura, F.; Ahmed, M.; Duk, S.; Rimpo, J.; Margolin, B. H.; Resnick, M. A.; Anderson, B.; Zeiger, E. Environ Mol Mutagen 1987, 10 (Suppl 10), 1.
62. Kawai, A.; Goto, S.; Matsumoto, Y.; Matsushita, H. Sangyo Igaku 1987, 29, 34.
63. Roberts, J. D.; Casserio, M. C. Basic Principles of Organic Chemistry; W.A. Benjamin, Inc.: New York, 1964.
64. Subrahmanyam, V. V.; Kolachana, P.; Smith, M. T. Arch Biochem Biophys 1991, 286, 76.
65. Hochstein, P. Fundam Appl Toxicol 1983, 3, 215.
66. King, C. M., Ed. Carcinogenic and Mutagenic Responses to Aromatic Amines and Nitroarenes; Elsevier: New York, 1988.
67. Sabbioni, G. Chem Biol Interact 1992, 81, 91.
68. Ford, G. P.; Herman, P. S. Chem Biol Interact 1992, 81, 1.
69. Novak, M.; Kennedy, S. A. J Am Chem Soc 1995, 117, 574.
70. Program Minitab 14. Available at: http://www.minitab.com/.
71. Guengerich, F. P. In Cytochrome P450: Structure, Mechanism, and Biochemistry; Ortiz de Montellano P. R., Ed.; Kluwer Academic/Plenum Publishers: New York, 2005; pp 377.
72. Schoch, G. A.; Yano, J. K.; Wester, M. R.; Griffin, K. J.; Stout, C. D.; Johnson, E. F. J Biol Chem 2004, 279, 9497.
73. Schoch, G. A.; Yano, J. K.; Sansen, S.; Dansette, P. M.; Stout, C. D.; Johnson, E. F. J Biol Chem 2008, 283, 17227.
74. Sansen, S.; Yano, J. K.; Reynald, R. L.; Schoch, G. A.; Griffin, K. J.; Stout, C. D.; Johnson, E. F. J Biol Chem 2007, 282, 14348.