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Prediction of Toxicity of Nitroaromatic Compounds through Their Molecular Structures

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In this paper, a new simple method is presented for the estimation of the toxicity of nitroaromatic compounds including some well-known explosives. This method can predict the 50% lethal dose concentration for rats (LD_{50}) as the estimation of toxicity *in vivo*. The prediction of LD_{50} of nitroaromatics through a new general correlation is based on the number of alkyl and nitro groups per molecular weight of the nitroaromatic compound as a core function. The existence of some specific structural parameters can decrease or increase the predicted results on the basis of the core function. The predicted results of various nitroaromatic compounds afford reliable prediction of LD_{50} with respect to experimental data. Prediction of toxicity for 28 nitroaromatic compounds, where the experimental data were available, and new nitroaromatic derivatives produce comparable results to those of several models of Quantitative Structure Activity Relation (QSAR).

Keywords: Correlation, Molecular structure, Nitroaromatic compound, Toxicity

INTRODUCTION

The risk, hazard and toxicity associated with the research investigations of nitro-compounds including high explosives can be reduced by the development of novel predictive methods. Safe handling of energetic compounds is one of the most important issues as far as scientists and engineers are concerned. It is important to understand the relationship between the specific stimuli, such as impact, friction, shock, electrostatic charge, heat, and the molecular structures of energetic molecules. Some simple correlations have been recently developed to predict impact, shock and electrostatic sensitivities of some selected classes of explosives [1-10].

Nitroaromatics are industrially produced chemicals [11] that can be used as solvents for synthesis of dyes, polymers and plastics or as bioactive products of insecticides, pesticides and pharmaceuticals [12,13]. They are also found as by-products of fuel combustion in vehicles and power plants [14]. They have different effects such as eco-toxic effects and life-threatening adverse drug reactions in humans. They display several manifestations of toxicity in humans, which include skin sensitization, immunotoxicity, and methaemoglobinemia [15]. Furthermore, high concentrations of nitroaromatic compounds in rats can cause liver, kidney and spleen toxicity. These adverse effects can be attributed to reactive metabolites by nitroreductase enzymes [12,16].

There are two basic approaches for the prediction of toxicity from chemical structure, *i.e.* the mechanistic approach and the statistical approach. In the mechanistic approach, a hypothesis is proposed that links a group of related chemicals with a particular toxicological endpoint. The hypothesis is then used to select physical, chemical or reactivity parameters to establish a Structure/Activity Relationship (SAR). The resulting relationship can be tested through redefining the

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hypothesis and parameters until an adequate predictive model is obtained [17]. In the statistical approach, a structure/activity association is generated between structural fragments or from (often large) numbers of computed parameters *via* these fragments. These systems use little or no expert judgment in organizing or selecting the data to be processed either on the basis of chemical class or by putative mechanism [18,19].

The toxicity of nitroaromatics has been the subject of a number of Quantitative Structure Activity Relation (QSAR) studies [20,21]. However, the studies have mostly concentrated on studies of acute toxicity towards aquatic species used for elucidating underlying modes of action and their link to the molecular structure of the compounds [20,21]. Only one study has been published to date that attempts to investigate toxicity factors occurring in mammals, which has compared rats *in vivo* lethal dose concentrations to various topological and quantum chemical descriptors [22].

Hierarchical Technology for Quantitative Structure-Activity Relationships (HiT QSAR) and 1D QSAR (one-dimensional) approaches were developed by Kusmin *et al*. [23,24]. They have predicted toxicity of twenty-eight nitroaromatic compounds including some well-known explosives. The LD_{50} was used as the estimation of toxicity *in vivo* to develop HiT QSAR and 1D QSAR models. The statistic characteristics for partial least squares 2D (two-dimensional) and 1D QSAR models were obtained with R^2 = 0.96-0.98 and 0.81-0.92, respectively. A QSAR analysis was developed by Agrawal *et al*. [25] on the toxicities of 40 mono-substituted nitrobenzenes using topological constitutional descriptors such as PI (Padmakar-Ivan index), Sz (Szeged index), MRI (molecular redundancy index) and J indices with R^2 = 0.74-0.76. The results have shown that no statistically significant mono-parametric QSAR models are possible. The predictive ability of the model was determined by a cross-validation method [25]. A Quantitative Structure-Property Relationship (QSPR) study was suggested for the prediction of toxicity of nitrobenzenes by Naizi and coworkers [26]. *Ab initio* theory has been used to calculate some quantum chemical descriptors including electrostatic potentials and local charges at each atom, *e.g*. HOMO and LUMO energies, *etc*. The correlation coefficients (R^2) was found to be 0.94 using MLR and PLS methods.

QSAR methods have some limitations such as: i) they are limited to the congeneric series; ii) large numbers of compounds are required to derive a good QSAR model; iii) they depend largely on the use of reliable data; and iv) QSAR for "predictive" purposes is not a realistic expectation [27,28].

The purpose of this work is to predict a reliable simple correlation for predicting the 50% lethal dose concentration for rats (LD_{50}) as the estimation of toxicity *in vivo* for different nitroaromatics. The new correlation is based on the molecular structure of nitroaromatics. The number of alkyl and nitro groups per molecular weight of the nitroaromatic compound are used as the core function. It shows how some specific structural parameters can decrease or increase the predicted results on the basis of the core function. The predicted results of training and test sets for various nitroaromatic compounds are compared to those of several models of QSAR. The novel method is also applied to different well-known aromatic energetic compounds and the predicted results are compared with those of the experimental data as well as the predicted values of QSAR models.

METHOD

Different QSAR models, descriptors and statistical methods have been used to develop toxicity models for diverse chemicals by different researchers, *e.g.* Xia *et al.* [29]. Rice and coworkers have reviewed [30] some studies that were conducted to develop QSARs or similar empirical correlations on the basis of molecular structure to predict potential hazards and properties of nitro compounds. The study of molecular structures of various nitroaromatic compounds with general formula $C_aH_bN_cO_d$ has shown that it is possible to get a reliable general correlation for predicting LD_{50} of these compounds. It was found that suitable combinations of the number of nitro and alkyl groups per molecular weight of the desired nitroaromatic as the core function (*F(core)*) as well as increasing (*F(incr)*) and decreasing (*F(decr)*) functions are important factors for the prediction of LD_{50}, as presented below.

$$-\log LD_{50}(/\,mmol\ kg^{-1}) = y_1 + y_2F(core) + y_3F(incr) + y_4F(decr) \quad (1)$$

where y_1 to y_4 are adjustable parameters that can be found from the experimental data given in Table 1. Multiple linear

Table 1. Comparison of the Predicted Values of -log(LD_{50}) by New Method with the Experimental Data [24] and the Predicted Results of Five Models of QSAR [24]

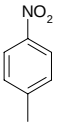
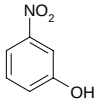
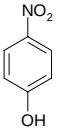
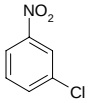
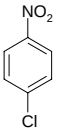
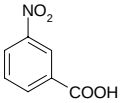
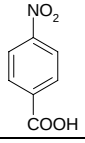
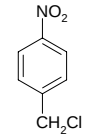
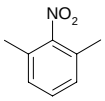
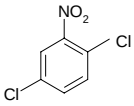
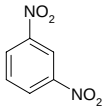
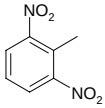
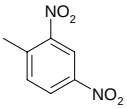
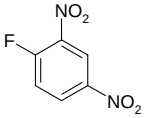
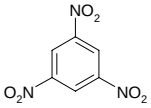
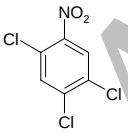
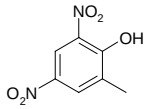
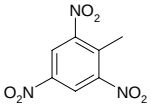
No.	Formula	*F*(*core*)	*F*(*decr*)	*F*(*incr*)	Obs.[a]	New[b] (Dev[c])	Model 1[d] (Dev)	Model 2[d] (Dev)	Model 3[d] (Dev)	Model 4[d] (Dev)	Model 5[d] (Dev)
1		0	1	0	-1.86	-1.85 (-0.01)	-1.73 (-0.13)	-1.76 (-0.10)	-1.68 (-0.18)	-1.65 (-0.21)	-1.71 (-0.15)
2		0.0109	0	0	-1.78	-1.83 (0.05)	-1.95 (0.17)	-1.94 (0.16)	-1.68 (-0.10)	-1.97 (0.19)	-1.89 (0.11)
3		-0.0081	1	0	-0.69	-0.82 (0.13)	-0.65 (-0.04)	-0.69 (0.00)	-0.84 (0.15)	-0.66 (-0.03)	-0.71 (0.02)
4		0	1	0	-1.19	-1.31 (0.12)	-1.02 (-0.17)	-1.01 (-0.18)	-1.08 (-0.11)	-1.16 (-0.03)	-1.07 (-0.12)
5		-0.0072	0	0	-0.38	-0.34 (-0.04)	-0.3 (-0.08)	-0.39 (0.01)	-0.45 (0.07)	-0.3 (-0.08)	-0.36 (-0.02)
6		-0.0072	0	0	-0.37	-0.34 (-0.03)	-0.34 (-0.03)	-0.22 (-0.15)	-0.15 (-0.22)	-0.42 (0.05)	-0.28 (-0.09)
7		-0.0072	0	1	-0.16	0.04 (-0.20)	-0.15 (-0.01)	-0.09 (-0.07)	-0.32 (0.16)	-0.16 (0.00)	-0.18 (0.02)
8		-0.0063	0	1	-0.23	-0.07 (-0.16)	-0.24 (0.01)	-0.56 (0.33)	-0.24 (0.01)	-0.52 (0.29)	-0.39 (0.16)
9		-0.0063	0	0	-0.39	-0.45 (0.06)	-0.36 (-0.03)	-0.54 (0.15)	-0.52 (0.13)	-0.53 (0.14)	-0.49 (0.10)
10		-0.0063	0	0	-0.43	-0.45 (0.02)	-0.55 (0.12)	-0.59 (0.16)	-0.52 (0.09)	-0.35 (-0.08)	-0.5 (0.07
11		-0.0060	0	0	-0.61	-0.50 (-0.11)	-0.84 (0.23)	-0.89 (0.28)	-0.75 (0.14)	-0.92 (0.31)	-0.85 (0.24)
12		-0.0060	1	0	-1.07	-1.09 (0.02)	-0.98 (-0.09)	-1.01 (-0.06)	-0.78 (-0.29)	-0.77 (-0.30)	-0.89 (-0.18)

Table 1. Continued

13		0	0	0	-1.02	-0.83 (-0.19)	-0.93 (-0.09)	-1.01 (-0.01)	-1.04 (0.02)	-0.92 (-0.10)	-0.98 (-0.04)
14		0.0066	0	0	-1.12	-1.12 (0.00)	-1.11 (-0.01)	-1.01 (-0.11)	-1.08 (-0.04)	-1.17 (0.05)	-1.09 (-0.03)
15		-0.0052	1	0	-1.32	-1.19 (-0.13)	-1.29 (-0.03)	-1.36 (0.04)	-1.24 (-0.08)	-1.27 (-0.05)	-1.29 (-0.03)
16		-0.0119	0	0	0.31	0.25 (0.06)	0.19 (0.12)	0.23 (0.08)	0.23 (0.08)	0.15 (0.16)	0.2 (0.11)
17		-0.0055	0	0	-0.14	-0.16 (0.02)	-0.09 (-0.05)	-0.08 (-0.06)	0.03 (-0.17)	-0.22 (0.08)	-0.09 (-0.05)
18		-0.0055	0	0	-0.17	-0.16 (-0.01)	-0.13 (-0.04)	-0.33 (0.16)	-0.12 (-0.05)	-0.3 (0.13)	-0.22 (0.05)
19		-0.0108	0	1	0.57	0.49 (0.08)	0.5 (0.07)	0.46 (0.11)	0.57 (0.00)	0.56 (0.01)	0.52 (0.05)
20		-0.0141	1	0	-0.11	-0.07 (-0.04)	-0.08 (-0.03)	-0.08 (-0.03)	-0.17 (0.06)	0.03 (-0.14)	-0.08 (-0.03)
21		-0.0044	0	0	-0.67	-0.70 (0.03)	-0.78 (0.11)	-0.61 (-0.06)	-0.97 (0.30)	-0.66 (-0.01)	-0.76 (0.09)
22		-0.0051	0	1	0.52	0.14 (0.38)	0.51 (0.01)	0.13 (0.39)	0.47 (0.05)	0.38 (0.14)	0.37 (0.15)
23		-0.0088	1	0	-0.49	-0.41 (-0.08)	-0.53 (0.04)	-0.53 (0.04)	-0.57 (0.08)	-0.41 (-0.08)	-0.51 (0.02)
rms deviation (mmol kg^{-1})						(0.12)	(0.08)	(0.10)	(0.12)	(0.11)	(0.07)

[a]Obs. is the observed value. [b]New is the predicted value using proposed model. [c]Dev = obs. - new. [d]Models 1 to 5 are predicted values using Kuz'min and coworkers [24].

regression method [29] has been used to find the adjustable parameters. Since the equation set is over-determined, the left-division method for solving linear equations uses the least squares method [29].

RESULTS AND DISCUSSION

The obtained results of multiple linear regression (MLR) method give the following optimized correlation:

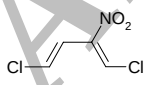
$$-\log LD_{50}(/\,mmol\ kg^{-1}) = -1.253 - 53.05F(core) - 0.5926F(decr) + 0.3853F(incr) \quad (2a)$$

$$F(core) = \frac{n_R - 2.38n_{NO_2}}{MW} \quad (2b)$$

where n_R and n_{NO2} are the number of alkyl and nitro groups attached to benzene ring. R-squared values or the coefficient of determination of Eq. (2) is 0.96 [33]. The values of *F(decr)* and *F(incr)* depend on some functional groups and specific substituents that can be specified as:

Prediction of *F(decr)*

(1) *Benzene and mononitro benzene derivatives*: For benzene and nitrobenzene, the value of *F(decr)* is equal to 1.0. For the presence of methyl and -COOH in para position to nitro group, *F(decr)* = 1.0. For the existence of two -Cl, where one of them is in *ortho* position of nitro group in the following form the value of *F(decr)* is also 1.0.



(2) *Benzene derivatives with* n_{NO2} = 3: The value of *F(decr)* is equal to 1.0 for those compounds that do not contain the attachment of potential donor fluorine atom and hydroxyl group to benzene ring.

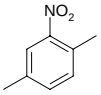
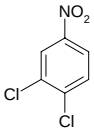
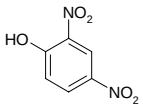
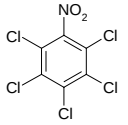
Prediction of *F(incr)*

(1) *Benzene derivatives with* n_{NO2} = 1: For the presence of -OH in para position to nitro group, *F(incr)* = 1.0. For the existence of one -Cl *ortho* to nitro group, the value of *F(incr)* is also 1.0.
(2) *Benzene derivatives with* n_{NO2} = 2: For the presence of -OH or -F *ortho* to nitro group, *F(incr)* = 1.0.

It should be mentioned that Eq. (2) is an empirical correlation, which was derived from the experimental data and foundations of QSAR of previous works [23,24]. The presence of some specific functional groups at particular positions attached to nitroaromatics or molecular fragments in the form of *F(decr)*and *F(incr)*, like the density of nitroaromatics [31,32], may correct the predicted results on the basis of *F(core)*. The increase of the number of nitro groups is important to enhance the toxicity because -NO_2 groups contain both nitrogen and oxygen atoms. It should be mentioned that the increase of the number of nitrogen and/or oxygen atoms in molecule is important to raise the toxicity. Since the nitrogen atoms used in the new correlation belong only to nitro group, we can conclude that increasing the number of nitro groups in the compound leads to the toxicity strengthening. The positive sign of n_{NO2} in Eq. (2) is indicative of this phenomenon. On the other hand, the insertion of the third nitro group into the aromatic ring inhibits toxicity of the investigated compound. Division of n_{NO2} by *MW* can retard the additive effect in the new correlation. Thus, the opposite effects of these parameters on toxicity show that the toxicity of trinitroaromatics is not higher than the toxicity of dinitroaromatics. According to the experimental data, an increase in the number of alkyl substituents, *e.g.* methyl and chlorine methyl in the compounds, is associated with a decrease in toxicity. This effect has been indicated in Eq. (2) through negative sign n_R. The introduction of fluorine atoms into benzene ring results in an increase of toxicity, whose effects were indicated in the above conditions for *F(decr)* and *F(incr)*.

The constants *F(decr)* and *F(incr)* are equal to zero if the conditions for giving them various values are not met. Twenty-three experimental data in Table 1 were used to get a new correlation. To test the new correlation, the calculated results for five nitroaromatics, where measured data were available, are also given in Table 2. Predicted results of five models of Kuz'min and coworkers [24] have also been given in Tables 1 and 2. As seen in these tables, the observed (Obs.), predicted (New) and deviations of predicted results from the observed values of LD_{50} are given. Moreover, toxicity has been predicted for 41 novel compounds the results of which are given in Table 3. The predicted results of five models of Kuz'min and coworkers [24] have also been given in Table 3.

Table 2. Comparison of the Predicted Values of -log(LD_{50}) by New Method with the Experimental Data [24] and the Predicted Results of Five Models of QSAR [24] (Test of New Correlation)

No.	Formula	*F*(*core*)	*F*(*decr*)	*F*(*incr*)	Obs.[a]	New[b] (Dev[c])	Model 1[d] (Dev)	Model 2[d] (Dev)	Model 3[d] (Dev)	Model 4[d] (Dev)	Model 5[d] (Dev)
1		0	0	0	-0.81	-0.72 (-0.09)	-0.92 (0.11)	-0.94 (0.13)	-0.96 (0.15)	-0.86 (0.05)	-0.92 (0.11)
2		0.0066	0	0	-1.21	-1.12 (-0.09)	-1.1 (-0.11)	-1.14 (-0.07)	-1.16 (-0.05)	-1.16 (-0.05)	-1.14 (-0.07)
3		-0.0052	0	0	-0.52	-0.60 (0.08)	-0.49 (-0.03)	-0.46 (-0.06)	-0.64 (0.12)	-0.41 (-0.11)	-0.5 (-0.02)
4		-0.0109	0	1	0.41	0.50 (-0.09)	0.5 (-0.09)	0.56 (-0.15)	0.57 (-0.16)	0.62 (-0.21)	0.56 (-0.15)
5		-0.0034	0	0	-0.57	-0.83 (0.26)	-0.63 (0.06)	-0.49 (-0.08)	-0.43 (-0.14)	-0.68 (0.11)	-0.56 (-0.01)
rms deviation (mmol kg^{-1})						(0.09)	(0.07)	(0.12)	(0.11)	(0.12)	(0.08)

[a]Obs. is the observed value. [b]New is the predicted value using proposed model. [c]Dev = obs. - new. [d]Models 1 to 5 are predicted values using Kuz'min and coworkers [24].

Table 3. Comparison of the Predicted Values of -log(LD_{50}) by New Method with the Predicted Results of Five Models of QSAR [24]

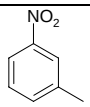
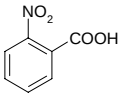
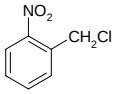
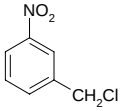
No.	Formula	F(core)	F(decr)	F(incr)	New	Model 1[a]	Model 2[a]	Model 3[a]	Model 4[a]	Model 5[a]
1		0	0	0	-0.72	-0.86	-0.89	-1.04	-1.13	-0.97
2		-0.0060	0	0	-0.50	-0.92	-0.94	-0.5	-0.7	-0.77
3		0	0	0	-0.83	-0.92	-0.94	-0.96	-0.84	-0.92
4		0	0	0	-0.83	-0.83	-0.89	-1.04	-0.9	-0.92

Table 3. Continued

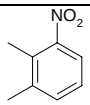
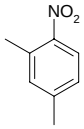
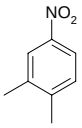
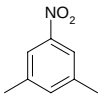
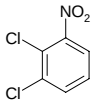
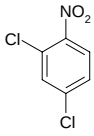
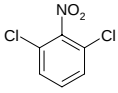
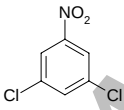
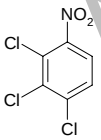
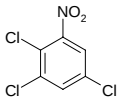
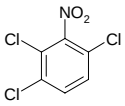
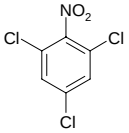
5		0.0066	0	0	-1.12	-1.1	-1.14	-1.16	-1.14	-1.14
6		0.0066	0	0	-1.12	-1.06	-1.08	-1.18	-1.14	-1.12
7		0.0066	0	0	-1.12	-1.2	-1.21	-1.28	-1.63	-1.33
8		0.0066	0	0	-1.12	-0.84	-0.91	-1.24	-1.56	-1.14
9		-0.0052	0	0	-0.60	-0.23	-0.37	-0.08	-0.61	-0.32
10		-0.0052	0	0	-0.60	-0.53	-0.58	-2.2	-0.6	-0.98
11		-0.0052	0	0	-0.60	0.05	-0.48	0.15	-0.6	-0.22
12		-0.0052	0	0	-0.59	-0.84	-0.62	-2.63	-0.85	-1.24
13		-0.0044	0	0	-0.70	-0.46	-0.4	-0.27	-0.49	-0.41
14		-0.0044	0	0	-0.70	-1.47	-0.81	-2.52	-1.59	-1.6
15		-0.0044	0	0	-0.70	-0.55	-0.34	-0.05	-0.74	-0.42
16		-0.0044	0	0	-0.70	-0.64	-0.62	-4.08	-0.91	-1.56

Table 3. Continued

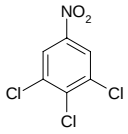
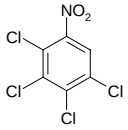
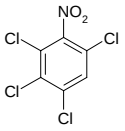
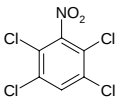
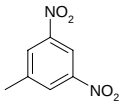
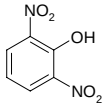
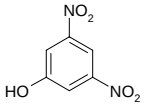
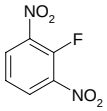
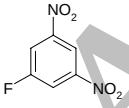
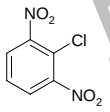
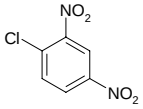
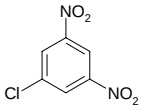
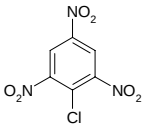
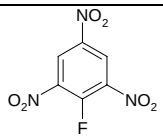
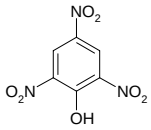
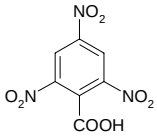
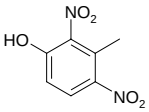
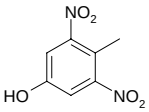
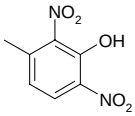
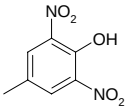
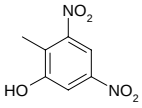
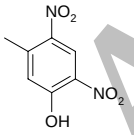
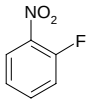
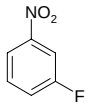
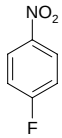
17		-0.0044	0	0	-0.70	-0.57	-0.75	-0.96	-0.47	-0.69
18		-0.0038	0	0	-0.77	-0.85	-0.92	-0.75	-0.54	-0.77
19		-0.0038	0	0	-0.77	-0.57	-0.27	-0.65	-0.79	-0.57
20		-0.0038	0	0	-0.77	-0.72	-0.19	-0.65	-1.06	-0.66
21		-0.0055	0	0	-0.16	0.01	0.02	-0.17	-0.19	-0.08
22		-0.0109	0	1	0.50	0.47	0.68	0.44	0.57	0.54
23		-0.0109	0	0	0.12	-0.29	-0.08	0.43	-0.25	-0.05
24		-0.0108	0	1	0.49	0.46	0.48	0.44	0.51	0.47
25		-0.0108	0	0	0.10	-0.27	-0.08	0.22	-0.25	-0.1
26		-0.0099	0	0	-0.01	0.37	0.35	0.55	0.13	0.35
27		-0.0099	0	0	-0.01	0.02	-0.05	0.27	0.26	0.13
28		-0.0099	0	0	-0.01	-0.44	-0.35	-0.57	-0.42	-0.45
29		-0.0121	1	0	-0.31	-0.42	-0.36	-0.36	0.56	-0.15

Table 3. Continued

30		-0.0130	0	0	0.39	0.26	0.52	0.04	1.01	0.46
31		-0.0131	0	0	0.40	0.23	0.71	0.04	1.08	0.52
32		-0.0122	1	0	-0.30	-0.46	-0.53	-0.57	-0.47	-0.51
33		-0.0051	0	1	0.14	0.22	0.33	0.37	0.36	0.32
34		-0.0051	0	0	-0.25	-0.65	-0.39	0.23	-0.6	-0.35
35		-0.0051	0	1	0.14	0.17	0.21	0.09	0.23	0.18
36		-0.0051	0	1	0.14	0.29	0.41	0.04	0.34	0.27
37		-0.0051	0	0	-0.25	-0.17	-0.46	0.13	-0.54	-0.26
38		-0.0051	0	1	0.14	0.49	0.08	0.27	0.22	0.27
39		-0.0071	0	0	-0.36	-0.30	-0.49	-0.45	-0.28	-0.38
40		-0.0071	0	0	-0.36	-0.34	-0.22	-0.25	-0.40	-0.30
41		-0.0071	0	0	-0.36	-0.20	-0.09	-0.32	-0.19	-0.20

[a]Models 1 to 5 are predicted values using Kuz'min and coworkers [24].

The predicted results of the new correlation are close to the experimental data and five complex models of Kuz'min and coworkers [24]. By considering the toxicity changes within the separate groups of mono-, di- and trinitrobenzenes of 69 related nitroaromatic compounds, the following remarks could be made:

(i) There was an increase in toxicity during the transition from mononitrobenzene derivatives to dinitrobenzene ones, probably because of their fast reduction to toxic intermediate [34]. Dinitrobenzenes as a group were more cytotoxic than mono-substituted nitrobenzenes towards rat hepatocytes. Meanwhile, a subsequent transition to substituted trinitrobenzene did not change toxicity appreciably.

(ii) Regarding mono-nitrobenzene, *para*-isomers had different effects on *F(decr)* and *F(incr)*.

(iii) There were different effects of chlorine atoms on n_{NO2} = 1, which are shown in *F(decr)* and *F(incr)*. Insertion of one chlorine atom in *ortho* position of -NO_2 group may increase toxicity, whereas insertion of the second chlorine atom in *para* position to the first one may decrease toxicity.

(iv) An independent analysis of nitrobenzene subgroups resulted in more robust linear-regression relationships and interpretations. The accumulation of chlorine atoms in the benzene ring results in the inhibition of their influence on toxicity. Furthermore, the most and least toxic chlorine-substituted nitrobenzenes contain the chlorine atom in *ortho* position to -NO_2 group. Previous studies postulated that mononitroaromatics with substituents in the *para* (*p*) position differed in mechanism from those with *ortho*- or *meta*-substituents [35]. It was suggested that a substituent in the *para* position hinders binding to the same receptor site [35] and has a stabilizing effect on the nitro group [36]. In molecule-containing aromatic amines, a sulfonic acid group has been shown to inhibit metabolic activation [37]. Such a procedure could be applied to nitrobenzenes.

(v) In most cases, insertion of fluorine atom and hydroxyl group into nitroaromatics can increase toxicity.

(vi) Due to the presence of multiple nitro groups, dinitrobenzenes are more toxic. The nitro group exerts an electron withdrawing effect on the phenyl ring, thus the phenyl ring is positively charged, while the nitro group has a negative charge. An increase in the number of nitro groups results in the increase in positive charge on the phenyl ring, hence a more electrophilic compound [21].

(vii) The opposite effects of the mentioned fragments on toxicity confirm that the toxicity of trinitroaromatics is not higher than that of dinitroaromatics. Such results, in turn, are indicative of the non-additive character of the simplex approach. In the investigated molecules, an increase in the number of carbon atoms (C_4 and C_6Cl fragments) was associated with a decrease in toxicity. The introduction of alkyl, specifically methyl and chlorine alkyl (for example, CH_2Cl) substituents, leads to a decrease in toxicity, whereas the introduction of fluorine atoms into the benzene ring (H_2F and CH_2F fragments) results in an increase in toxicity.

Toxicity of nitroaromatics is affected by interface of the substituents. Equation (2) was derived in a situation where all the variables had non-additive contributions for substituents. The predicted results confirm this and indicate that toxicity of substituents to the benzene ring is considerably non-additive.

The basic statistical parameters such as standard error of the estimate, standard error of the regression coefficient and adjusted R-squared are calculated and summarized in Table 4. External validation was used to study the validity of the proposed model [38,39]. The purpose of validation was to provide a statistically reliable model with selected descriptors as a consequence of the cause-effect and not only of pure numerical relationship obtained by chance. The values of *F(core)* on the basis of Eq. (2b) as well as *F(decr)* and *F(incr)* according to conditions of (a) and (b) are given in Tables 1-3.

A modified r^2 term (r^2_m) was calculated to better indicate the external predictive capacity of the model (Table 4). The magnitude r^2_o represents the squared correlation coefficient between the observed and predicted values of the test set compounds setting the intercept to zero. Note that r^2 is always larger than r^2_o. In case of good external prediction, the predicted values would be very close to the observed activity values. So, the r^2 value would be very close to the r^2_o value. In the best case, r^2_m would be equal to r^2 whereas in the worst case r^2_m value would be zero [40].

CONCLUSIONS

A simple correlation was used to predict toxicity (LD_{50} values) of nitroaromatic compounds. The proposed equation was derived on the basis of the number of alkyl and nitro

Table 4. Statistical Analysis of Data of Table 1 in Deriving Eq. (2)

Parameter	Eqation	Result
Regression coefficient		$R^2 = 0.9628$
Adjusted R-squared	$R^2_{adj} = 1-\left[(1-R^2)(n-1)/(n-k-1)\right]$	0.9497
Standard error of the regression coefficient	$S_b = \frac{S_e}{\left[\sum (Y_{obs} - \overline{Y}_{Table\ 1})^2\right]^{1/2}}$	0.0384
Standard error of the estimate	$S_e = \left[\sum (Y_{obs} - Y_{pred})^2/(n-k-1)\right]^{1/2}$	0.14127
Predictive coefficient R^2_{pred}	$R^2_{pred} = 1-\frac{\sum (Y_{obs} - Y_{pred})^2}{\sum (Y_{obs} - Y_{Table\ 1})^2}$	0.9323
Modified r^2 (r^2_m)	$r^2_m = r^2\left(1-\sqrt{r^2 - r^2_o}\right)$	0.8106

groups per molecular weight of the nitroaromatic compound as a core function. The present method, apart from being a predictive tool, can be used as the simplest procedure for the prediction of toxicity of nitroaromatic compounds including high explosives. Moreover, predictions made by the proposed method can be reasonably reliable compared to those of the complex QSAR computations. The suggested method is quite appealing and promising because it requires as input only structural formula of nitroaromatics.

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