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Partial Order Ranking for the aqueous toxicity of aromatic mixtures

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Abstract We apply a predictive method based on Partial Order Ranking that employs a single molecular descriptor in the model and that is simple enough to perform calculations by hand. A comparison of this procedure with results obtained from the least squares technique is carried out, using aqueous toxicity values elicited by 67 compounds and their aromatic mixtures, and the octanol/water partition coefficient as structural descriptor. Both techniques verify that, by means of a previous classification of the compounds in polar and non-polar groups, it is possible to predict the joint toxicological effect.

Keywords QSPR–QSAR theory · Least Squares method · Partial Order Ranking ·
Vibrio fischeri · Octanol/water partition coefficient

1 Introduction

Aromatic derivatives are characterized for their usefulness as intermediaries in the synthesis of commercial products such as pesticides, herbicides, plastic products, and also for the molecular design of drugs or other organic compounds. Nevertheless, it is necessary to evaluate a priori the toxicological adverse effects that some chemical agents produce when liberated into the environment, along with some convenient way to control their production [1]. This is not an easy task, given that an intensive toxicological test is usually expensive, besides being a time demanding process. Furthermore, a study of this nature should be able to consider multiple environments and

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the effect of every possible combination of biological interactions over the diverse living organisms which are representative of the different ecosystems, information that is not always available [2]. In recent times, this issue has raised the concern of thousands of scientists worldwide, which advocated themselves to the study of the behaviour of suspiciously dangerous substances in various fields such as Environmental Chemistry or Toxicology [3].

Whenever it is not possible to perform intensive biological tests over complex systems, applying semiempirical or theoretical methodologies proves to be an adequate alternative way for obtaining information about the eco-toxicological features of a given compound. The different formulations of the Quantitative Structure–Activity Relationships (QSAR) offer mathematical models which would be able to quantify a hypothetical unknown relationship between a substance's molecular structure and its in vivo/in vitro toxicity. A set of numerical descriptors [4] describing the molecular structure are calculated, usually representing physicochemical properties experimentally determined or theoretical quantities derived from different theories, such as the Graph Chemical Theory [5]. Recently, Cronin et al. [6] and Comber et al. [7] reviewed on the use of QSAR by regulative authorities in order to predict water toxicity, mutagenesis, carcinogenesis and other adverse effects.

In general, when biological properties are modelled by QSAR it is possible to classify the models either as mechanistic or statistical [8]. A mechanistic model is one which takes into account the biophysical interaction mechanism by which a given chemical compound acts on the ecosystem; a statistical model does not consider the mechanism, but tries to get the best predictions by searching for the best model. A lot of experimental work is required in order to determine a toxicological mode of action [9], and many times the selection of a determined model lacks precise and reproducible experimental evidence, consequently forcing one to apply intuition [10]. Various efficient methodologies have been proposed to describe toxicological modes of compounds from the knowledge of their molecular structures [11]. The issue can be complicated by factors such as the existence of more than one intervenient mechanism [12], transformation of the species [13,14], and changes of the mechanism even through an homologue molecular series [15,16]. Usually, QSAR models are designed for compounds which offer the same toxicological mode of action, since this conducts to more accurate predictions [17].

It is known that when a malfunction of the cell membranes in a biological organism is present, a narcotic mode of action occurs. Within the family of organic compounds, it is feasible to experimentally identify modes of action as non-polar-narcotics or baseline-narcotics [18] and polar-narcotics or electro(nucleo)philic [19]. A non-polar narcotic mode of action can be explained by an adjustment by hydrophobicity descriptors such as $\log K_{ow}$. This parameter represents important bio-effects such as diffusion through cell membranes [20]. When toxicity exceeds the amount associated with a non-polar narcotic action [21], it is considered that the molecule acts by means of a reactive electrophilic mechanism. In that circumstance, experience teaches that the property must be predicted by hydrophobicity type of descriptors along with other descriptors that take into account the spatial distribution of the electronic molecular charge, such as the energy of electrons in the lowest unoccupied molecular orbital (LUMO) within the context of the Molecular Orbital Theory [22,23].

For the special case when toxicity cannot be explained by any of these methods, or should it be an intermediate effect between both of them, then we would be in presence of a syndrome other than narcotic [24]. Nevertheless, most industrial organic compounds show toxicological modes of action of the narcotic type. Very often the mechanism of action depends not only on the compound structure but also on the specific environment upon which it is acting. From this perspective, a structural classification layout of the molecules would be useful since it reduces the number of alternatives to consider and allow a more focalized toxicity study. This is one reason for which in this work the calibration set of compounds is partitioned into polar and non-polar groups, depending on the type of functional groups present in each molecule.

The area of Aqueous Toxicology is considered to be well represented by the effects of chemicals over marine species such as *Vibrio fischeri* (*Tetrahymena pyriformis*) or *Fathead minnow* (*Pimephales promelas*) [13,25–27]. Even though a large variety of QSAR models on aqueous toxicity of single chemicals exist, little has been done to predict their combined effect [28–33]. In present study, 67 aromatic substituted compounds and their mixtures combining up to five components are analyzed, which inhibit the light emission of the ciliated marine bacteria *Vibrio fischeri*. For this purpose, we resort to a new method based on the technique of Partial Order Ranking and that employs the partition coefficient log K_{ow} as a single descriptor, and results are compared to the ones obtained via the Least Squares method reported in the study of Wei et al. [34]. Present article is organized as follows: next section describes the methods employed, then the results are shown and finally the main conclusions of this paper are summarized, along with possible extensions of the proposed methodology.

2 Method

The methodology of Partial Order Ranking [35–37] including a single molecular descriptor has an extremely simple principle: if a molecule J displaying a property p_J is characterized with a descriptor d_J, then two molecules A and B can be compared if and only if their descriptors can be compared. In other words,

$$p_B \leq p_A \leftrightarrow d_B \leq d_A \tag{1}$$

When the rule (1) is true then it is said that compound A is ranked higher than compound B. If (1) is false, then both B and A are incomparable. Note that (1) a priori includes "$\leq$" as the only structural function.

First of all consider a calibration set a with N compounds. The application of inequality (1) to this set will generate two different subsets a_1 and a_2: in a_1 all the molecules will satisfy (1) and the second subset a_2 will contain those compounds which do not follow the rule. However, if we apply again (1) to a_2 we will generate two new different subsets a_3 and a_4, with fewer elements each one, where compounds in a_3 are ordered and compounds in a_4 do not obey rule (1). Proceeding in this way again repeatedly, we continue iterating until the number of compounds in the second subset is zero. This condition is achieved only if the selected descriptor can describe

the whole calibration set. Otherwise, the second subset of the last iteration will not be empty. After all this procedure is done, we will have the following k ordered subsets $a_h/h = 1, \ldots, k$, and where k is dependent on the property p under consideration and the descriptor d employed.

In order to predict the property p_I of a given compound I with descriptor value d_I from the calibration set using the k ordered subsets, we can use simple interpolation formulae. Firstly, we have to locate the subset a_x that contains a compound J (with I excluded from a_x) that satisfies the next condition:

$$\text{absolute } (d_J - d_I) = \text{ minimum} \tag{2}$$

Once located the subset a_x and the molecule J, then the following situation will appear in a_x,

$$
\begin{array}{cc}
p_J & d_J \\
 & d_I \\
p_{J+1} & d_{J+1}
\end{array}
$$

where we have the ranking $J \leq I \leq J + 1$.

The linear interpolation formulae can be deduced as

$$p_J = \text{a}^* d_J$$
$$p_{J+1} = \text{a}^* d_{J+1}$$
$$p_I = \text{a}^* d_I$$
$$\frac{p_{J+1} - p_J}{d_{J+1} - d_J} = \text{a}$$
$$p_I^{pred} = \frac{p_{J+1} - p_J}{d_{J+1} - d_J} * d_I$$

with p_I^{pred} denoting the predicted value of p_I. For the special case where $p_j = p_{min}$ or $p_j = p_{max}$, where p_{min} and p_{max} are the minimum and maximum values of p_j in a_x, respectively, we can obtain from the previous equations an extrapolation formula,

$$p_J = a * d_J$$
$$\frac{p_J}{d_J} = \text{a}$$
$$p_I^{pred} = \frac{p_J}{d_J} * d_I$$

If we have a validation set we proceed in a similar way as indicated above: first, localizing the minimum difference between the descriptor d_I of the validation set and a d_J from a subset a_x according to the condition (2), and then applying the linear interpolation formulae. It can be shown that the lower the value of k the better are the estimations of the proposed methodology, since condition (2) is not sufficient to lead

to the best predictions for a descriptor d_I in a_x when k is greater than 1. Another point is the length of the interval $d_{J+1} - d_J$: the smaller the length of the interval, the better the predictions. This is in consequence of the linear interpolation formulae: a secant line is approximating a tangent line in a property versus single descriptor-graph.

3 Results and discussion

In Table 1 we show the experimental toxicity values measured for 34 aromatic substituted organic compounds and 33 mixtures of them, composed with up to five components: benzene, aniline, phenol, nitrobenzene and 4-chlorophenol. Aqueous toxicity is expressed as the necessary concentration for the inhibition at 50% of the bioluminescence after 15 min of exposition, EC_{50} (mg l^{-1}); it is converted into $-\log EC_{50}$ for modelling purposes. This table also includes the descriptor $\log K_{ow}$ employed previously [34], which in case of a mixture should depend on the concentrations, and is obtained by the semiempirical formulae proposed by Verhaar et al. [38], as follows:

$$K_{ow}^{mixture} = \frac{V_t}{V_o} * \frac{\sum_{i=1}^{n} \frac{Q_i}{1+(V_t/V_o^* K_{owi})}}{\sum_{i=1}^{n} Q_i - \sum_{i=1}^{n} \frac{Q_i}{1+(V_t/V_o^* K_{owi})}} \tag{3}$$

where $K_{ow}^{mixture}$ is the partition coefficient for the mixture, V_t is the total volume of the solution, V_o is the volume of the octanol phase, Q_i is the initial molar quantity of the i chemical in water, n is the total number of components in the mixture, and K_{owi} is the partition coefficient for the pure compound. In this paper $V_t/V_o = 6.8 \times 10^5$ was established, since as for a big value of this quotient, $K_{ow}^{mixture}$ remains independent of V_t/V_0.

In a previous work [34] it was pointed out that by making a previous classification of the compounds and their mixtures in polar and non-polar substances after the type of functional groups which appeared in them, it was possible to achieve models of better quality, at least for the statistical parameters of training and testing, when compared against not making that kind of classification. A mixture is considered non-polar it does not contain benzene as component. The models found with the least-square method for both groups of compounds are as follows:

Pure compounds and their polar mixtures:

$$- \log EC_{50} = -1.634 + 1.054 \log K_{ow}$$
$$N = 40, R = 0.965, S = 0.163, F = 521, rms = 0.0251, p < 10^{-4}$$
$$R_{leave-one-out} = 0.961, S_{leave-one-out} = 0.168 \tag{4}$$

Pure compounds and their non-polar mixtures:

$$- \log EC_{50} = -2.801 + 1.076 \log K_{ow}$$
$$N = 27, R = 0.969, S = 0.203, F = 387, rms = 0.0382, p < 10^{-4}$$
$$R_{leave-one-out} = 0.962, S_{leave-one-out} = 0.216 \tag{5}$$

Table 1 Experimental and predicted toxicity values by different QSAR models

No.	Sample	Ratio (mg l^{-1})	log K_{ow}	$-\log EC_{50}$	Eq. 4	Eq. 5	Eq. 6	Eq. 7
1	B	–	2.13	−0.95	–	−0.51	–	−0.70
2	Toluene	–	2.73	0.20	–	0.14	–	0.14
3	1,2-Xylene	–	3.12	0.45	–	0.56	–	0.50
4	1,3-Xylene	–	3.20	0.44	–	0.64	–	0.57
5	1,4-Xylene	–	3.15	0.53	–	0.59	–	0.48
6	4-Chlorotoluene	–	3.33	0.88	–	0.78	–	0.69
7	CB	–	2.84	0.30	–	0.26	–	0.26
8	1,2-Di-CB	–	3.43	0.78	–	0.89	–	1.12
9	1,3-Di-CB	–	3.53	0.74	–	1.00	–	1.26
10	1,4-Di-CB	–	3.44	1.15	–	0.90	–	0.80
11	1,2,3-Tri-CB	–	4.05	1.54	–	1.55	–	1.92
12	1,2,4-Tri-CB	–	4.02	1.91	–	1.52	–	1.49
13	4-CA	–	1.83	0.57	0.29	–	0.70	–
14	2-CP	–	2.15	0.55	0.63	–	0.78	–
15	3-CP	–	2.50	0.96	1.00	–	1.07	–
16	2,3-Di-CP	–	2.84	1.52	1.35	–	1.13	–
17	2,4-Di-CP	–	3.06	1.47	1.59	–	1.24	–
18	2,5-Di-CP	–	3.06	1.24	1.59	–	1.47	–
19	2,6-Di-CP	–	2.75	1.09	1.26	–	1.40	–
20	2-Nitro-A	–	1.85	0.71	0.32	–	0.28	–
21	2-Nitro-P	–	1.79	0.53	0.25	–	0.19	–
22	3-Nitro-P	–	2.00	0.34	0.47	–	0.47	–
23	2-Nitro-CB	–	2.24	0.97	0.73	–	0.89	–
24	3-Nitro-CB	–	2.46	1.05	0.96	–	0.78	–
25	4-Nitro-CB	–	2.39	0.94	0.88	–	0.89	–
26	2-Nitro-toluene	–	2.30	0.91	0.79	–	1.00	–
27	3-Nitro-toluene	–	2.45	0.74	0.95	–	1.02	–
28	4-Nitro-toluene	–	2.37	0.90	0.86	–	0.88	–
29	2-Methyl-P	–	1.95	0.23	0.42	–	0.34	–
30	3-Methyl-P	–	1.96	0.35	0.43	–	0.25	–
31	P	–	1.46	−0.04	−0.10	–	−0.17	–
32	A	–	0.98	−0.65	−0.60	–	−0.65	–
33	NB	–	1.87	0.30	0.34	–	0.72	–
34	4-CP	–	2.39	0.89	0.88	–	0.94	–
35	B+A	Equitox	1.97	−0.75	–	−0.68	–	−0.40
36	B+P	Equitox	2.09	−0.79	–	−0.55	–	−0.48
37	B+NB	Equitox	2.12	−0.65	–	−0.52	–	−0.40
38	B+4-CP	Equitox	2.14	−0.70	–	−0.50	–	−0.93
39	A+NB	Equitox	1.21	−0.42	−0.36	–	−0.31	–
40	A+4-CP	Equitox	1.21	−0.31	−0.36	–	−0.42	–
41	P+4-CP	Equitox	1.72	0.13	0.18	–	0.12	–
42	P+A	Equitox	1.12	−0.59	−0.45	–	−0.68	–
43	NB+P	Equitox	1.63	0.00	0.08	–	0.32	–
44	NB+4-CP	Equitox	2.08	0.54	0.56	–	0.72	–
45	B+A+NB	Equitox	1.96	−0.66	–	−0.69	–	−0.75
46	B+A+P	Equitox	1.94	−0.70	–	−0.71	–	−0.61
47	B+A+4-CP	Equitox	1.57	−0.68	–	−1.11	–	−0.57
48	B+NB+4-CP	Equitox	2.12	−0.40	–	−0.52	–	−0.65
49	B+P+4-CP	Equitox	2.10	−0.53	–	−0.54	–	−0.74
50	B+P+NB	Equitox	2.08	−0.78	–	−0.56	–	−0.79
51	P+A+4-CP	Equitox	1.27	−0.38	−0.30	–	−0.30	–
52	P+NB+4-CP	Equitox	1.77	0.18	0.23	–	0.51	–
53	A+NB+P	Equitox	1.26	−0.32	−0.31	–	−0.39	–

Table 1 continued

No.	Sample	Ratio (mg l^{-1})	log K_{ow}	$-$log EC_{50}	Eq. 4	Eq. 5	Eq. 6	Eq. 7
54	A+NB+4-CP	Equitox	1.34	−0.30	−0.22	–	−0.17	–
55	B+A+P+NB	Equitox	1.94	−0.61	–	−0.71	–	−0.70
56	B+A+P+4-CP	Equitox	1.95	−0.60	–	−0.70	–	−0.65
57	B+A+NB+4-CP	Equitox	1.97	−0.40	–	−0.68	–	−0.75
58	B+P+NB+4-CP	Equitox	2.09	−0.48	–	−0.55	–	−0.79
59	A+P+NB+4-CP	Equitox	1.36	−0.13	−0.20	–	−0.27	–
60	B+A+P+NB+4-CP	Equitox	1.95	−0.65	–	−0.70	–	−0.60
61	4-CP+NB	1:1	2.09	0.75	0.57	–	0.54	–
62	4-CP+NB	1:2	1.93	0.64	0.40	–	0.22	–
63	4-CP+NB	1:5	1.66	0.37	0.11	–	0.04	–
64	P+A	1:0.4	1.37	−0.26	−0.19	–	−0.12	–
65	P+A	1:1	1.28	−0.35	−0.29	–	−0.37	–
66	P+A	1:2	1.20	−0.47	−0.37	–	−0.31	–
67	P+A	1:5	1.10	−0.73	−0.48	–	−0.58	–

B: benzene, P: phenol, A: aniline, CP: chlorophenol, NB: nitrobenzene, CB: chlorobenzene, CA: chloroaniline, equitox: equitoxicity

in which the statistical parameters R, S, and F represent the correlation coefficient, standard deviation of the model and Fisher ratio of the training set, respectively, whereas *rms* denotes the model's root mean squared deviation and p is the significance of the model. $R_{leave\text{-}one\text{-}out}$ and $S_{leave\text{-}one\text{-}out}$ are the correlation coefficient and standard deviation obtained with the Leave-One-Out Cross-Validation method and provides information on the model's predictive strength [39].

Applying now the Partial Order Ranking method we find the predictions for the polar and non-polar groups by interpolation, along with the indicated values of k. In order to make a comparison with the previous models, a correlation between the values predicted with this method and the properties observed is made:

Pure compounds and their polar mixtures ($k = 5$)

$$-\log EC_{50} = 0.012 + 0.948 \log K_{ow}$$
$$N = 40, R = 0.937, S = 0.219, F = 271, rms = 0.0453, p < 10^{-4} \quad (6)$$

Pure compounds and their non-polar mixtures ($k = 7$)

$$-\log EC_{50} = 0.048 + 0.059 \log K_{ow}$$
$$N = 27, R = 0.953, S = 0.251, F = 245, rms = 0.0583, p < 10^{-4} \quad (7)$$

From considering the minimization of the standard deviation for the training and test sets as a measure for the model's quality, it is concluded that the partial order method generates worse predictions. This result is explained by the fact that descriptor K_{ow} is not the optimal one for ranking the molecules. Whenever a descriptor works well in a regression, this won't necessarily mean that it will work as well in Partial Order Ranking. An analogue situation is also valid: should a descriptor order as the property does, it won't always conduct to a good correlation with the property.

If both polar and non-polar substances are grouped and modelled simultaneously with the partial order strategy, it is observed that the results get even worse, suggesting that the polar-non-polar classification scheme is an efficient way to improve the model's accuracy, which is in line with previous findings obtained through other methodology, i.e. linear regressions [34]:

Pure compounds and their mixtures

$$-\log EC_{50} = 0.110 + 0.685 \log K_{ow}$$
$$N = 67, R = 0.713, S = 0.510, F = 67.060, rms = 0.252 \qquad (8)$$

The nature of the new proposed method reveals two main points:

(i) Given that (1) includes "$\leq$" playing the role of relating the numerical data, this will hold whenever the descriptor follows the rule. This means that, in comparison with the classical least square criterion, there is no need to search for a complicated functional form of the model for those descriptors which do not behave adequately. This is the main utility of the proposed technique.

(ii) The method does not need to be validated by means of the Leave-One-Out Cross Validation technique. Actually, when the dependent property of compound i is being predicted by the interpolation formulae, the Leave-One-Out technique is in fact being applied, given that compound i is being left outside the training set while being predicted by their immediate neighbours, j and $j + 1$. A leave-one-out technique like this should work better than the leave-one-out commonly used in the Least Square method, as it does not depend on the functional form of the model anyway. In other words, partial order's R and S parameters represent the $R_{leave-one-out}$ and $S_{leave-one-out}$ of the Least Square method.

4 Conclusions

It was verified after applying the Partial Order Ranking method that a previous classification scheme for pure compounds and their mixtures in polar and non-polar groups allows the simultaneous modelling of both groups. Furthermore, the results obtained here point out to the predictive capability of the proposed technique and anticipate its main advantages and deficiencies for any future application. The Partial Order Ranking method does not need to specify a mathematical function for the model, a desirable feature for those cases where the dependent property change in a complex way with the molecular structure. In order to further value the characteristics of this procedure, it should be taken into account that it consists only on sorting data and performing linear interpolations.

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