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Some findings relevant to the mechanistic interpretation in the case of predictive models for carcinogenicity based on the counter propagation artificial neural network

Natalja Fjodorova · Marjana Novič

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Abstract The goal of the study was to contribute to a better mechanistic understanding of so-called "general" QSAR models for non-congeneric chemicals based on the counter propagation artificial neural network (CP ANN). Possible mechanisms of action was proofed using the Toxtree expert system based on structural alerts (SAs) for carcinogenicity. We have illustrated how statistically selected MDL descriptors, which refer to topological characteristics as well as to polarizability and charge distribution related to reactivity, are correlated with particular chemical classes (containing carcinogenic SA) with the recognized mechanistic link to the carcinogenic activity and consequently with the carcinogenic potency. Mechanistic insight in CP ANN models was demonstrated using an inherent mapping technique (i.e. Kohonen maps).

Keywords Counter propagation artificial neural network · Mechanistic interpretation of model · Carcinogenicity · QSAR model · Structural alerts · Chemical descriptors · Kohonen maps · Non-congeneric chemicals · Classification model

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N. Fjodorova (✉) · M. Novič
National Institute of Chemistry, Hajdrihova 19,
SI-1001 Ljubljana, Slovenia
e-mail: natalja.fjodorova@ki.si

M. Novič
e-mail: marjana.novic@ki.si

Introduction

According to the OECD Member Countries and the European Commission agreement a (quantitative) structure–activity relationships ((Q)SARs) model should follow five principles to establish the scientific validity and its acceptance for regulatory purposes. One of these principles should be associated with mechanistic interpretation if possible [1]. The mechanistic understanding of models is very important for risk assessment to ensure the safety of chemicals. In the modelling the mechanistic association between descriptors and the endpoint should be considered.

The mechanistic investigation related to structure activity relationships of chemical mutagens and carcinogens was reported in several papers [2–7]. The study of individual QSAR models for congeneric series of chemicals (aromatic amines, nitroaromatic compounds, *N*-nitroso compounds, polycyclic aromatic hydrocarbons and etc.) showed that in most cases electronic properties (HOMO, LUMO) together with steric ones (size/shape) appeared to determine the minimum requirement for the chemicals to be metabolised and to differentiate the active from inactive ones, while hydrophobicity (logP) determined the extent of activity i.e. the potency of active compounds. The application of QSAR modeling to individual classes of chemicals showed good predictive ability and understanding the biological activity (mutagenicity and carcinogenicity) and provided information on the mechanisms of action depending on the relevant properties/features of the chemicals. On the other hand the individual QSAR models are limited by using only certain class of chemicals [3].

Recently, the need for setting the priority of chemicals due to the chemical regulation and tendency for reducing animal testing has motivated the development of QSAR models for non-congeneric set of chemicals (so-called

"general" QSARs) that are able to predict carcinogenicity for a wide diversity of molecular structures, spanning an undetermined number of chemical classes and biological mechanisms. The most of "general" QSAR models focused on the predicting rodent carcinogenicity. In the case of non-congeneric chemicals multiple mechanisms of action can lead to the same toxicity endpoint. The QSAR model for non-congeneric chemicals has to model the various mechanisms of action of various types of chemicals present in a studied data set. As a matter of fact, all "general" models consist of multiple local models, whose definition may or may not be readily apparent [4]. Hence, the "general" approaches for non-congeneric chemicals are able to provide little or no mechanistic information. On the other hand they are able to generate correct predictions of activity for untested chemicals. Therefore, the mechanistic insight into a "general" model is of great interest nowadays.

Different approaches related to the "general" carcinogenicity models were described in the papers [3, 5]. Generally, they can be divided into rule-based and statistically-based methods. The rule-based (or knowledge-based) methods combine toxicological knowledge, expert judgment and fuzzy logic. The software such as Hazard-Expert, OncoLogic, Toxtree, and DEREK attempt to codify the existing knowledge, derived from the human expert judgment, bioassay data, or any of modeling approaches, into generalized rules to be used in a prediction. The following data relevant to the carcinogenic potency are considered: toxicokinetics and toxicodinamics parameters that affect the delivery of biologically active intermediates to target tissues for interaction with cellular macromolecules or receptors. In contrast, the statistically-based methods (i.e. statistical, multivariate, rule-induction, artificial intelligence, cluster analysis, pattern recognition and etc.) deal with limited or no prior chemical or biological classification according to mechanism. MultiCASE, Leadscope, TOPKAT, LAZAR and CAESAR belong to the statistically-based systems where discovery of genotoxic or chemical fragments (SAR knowledge) are identified by specific automated algorithms [6]. It is clear that each of approaches has the potentials and limitations. The knowledge-based approaches provide opportunity to gain insight into the mechanism underlying the mutagenicity/carcinogenicity whereas in the case of statistically based models it is usually difficult to interpret the models and to provide mechanistical reasoning of the predictions. On the other hand, not all the rule-based models can explain the differences of the activity within a chemical class. The main advantage of the statistically-based models is high accuracy of prediction whereas the rule-base models usually have lower accuracy of prediction than statistical ones [5].

Carcinogenicity models based on artificial neural network (ANN) were reported recently [8, 9]. The main advantage of ANN modelling of carcinogenicity is that complex, non-linear relationship can be modelled without any assumptions about form of the model. Large data set can be examined. ANNs are able to cope with noisy data and are fault tolerant [9]. On the other hand, ANNs act like "black boxes". The relationships are encoded as weight vectors within the trained network, which is difficult to explain in comprehensible form. The knowledge-based information embedded within ANNs can be extracted and expressed as a set of comprehensible data. Therefore, the knowledge-based Toxtree expert system (based on the decision tree approach) was employed as a supporting tool in interpretation of obtained results in terms of possible mechanism of action of studied chemicals. Such extracted information enhances the understanding studied prediction ANN model and enables the obtaining the mechanistic interpretation.

The counter propagation artificial neural network (CP ANN) model for prediction of carcinogenicity based on eight MDL descriptors was considered in the article. From statistical point of view the model showed good recall ability and acceptable accuracy of prediction (73%) as was reported in the article [8]. The mechanistic basis of the model was determined a posteriori (after the modeling), by interpretation of the final set of training structures and descriptors.

The mechanistic interpretation of the model in this study was determined using inherent to CP ANN mapping technique (Kohonen maps) which enable researcher to analyse the distribution of chemicals, individual descriptors (in weight level maps) and carcinogenic potency (Yes/No) in the same 2D space. The Toxtree expert system provided the information about classes of used chemicals [on the basis of carcinogenic structural alerts (SAs)] and their possible mechanisms of action. Thus, the model became transparent.

Data and method

Data used in the model

The dataset of 805 chemicals used for modeling was extracted from initial dataset of 1,481 chemicals taken from Distributed Structure-Searchable Toxicity (DSSTox) Public Database Network http://www.epa.gov/ncct/dsstox/sdf_cpdbas.html [10]. The carcinogenic potency for rats was used as a response. Additionally, for each chemical in the dataset we collected carcinogenic structural alerts (SAs) (if available) using Toxtree expert system [7]. The Toxtree represents the list of 33 SAs. Five of them refer to non-genotoxic mechanisms of action while others refer to genotoxic ones. The Toxtree rulebase was reported by

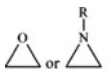
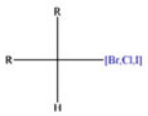
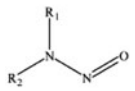
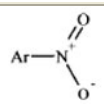
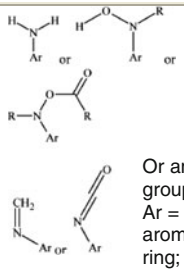
Benigni and Bossa [7]. Carcinogenic SAs are molecular substructures or functional groups that have been mechanistically and/or statistically associated with induction of cancer [11]. Therefore, the set of chemicals characterised by the same SA could compose a family of compounds with the same mechanism of action (in a broad sense). Table 1 containes the compilation of chemicals classes coded as SA with recognized mechanistic link to carcinogenicity. Two groups of chemicals from the studied dataset containing two SAs were also considered in the model.

Eight MDL descriptors that maximally explain the variance in observed carcinogenic potency (property or activity of interest) were applied in the model as was explained in the article [8]. The MDL QSAR version 2.2 software [12] generates descriptors derived from a molecular graph theory or topological representation of structure. The mechanistic interpretation of the model was made by examination of changes in key structural features identified by descriptors (that affect the observed properties of a studied molecule) in the context of model training set.

The CP ANN method

The CP ANN method used in present study is described in details in the recent paper [8]. It should be remarked that CP ANN belongs to self organizing map technique that often used to analyse the data in multi-dimensional space. Basis of this technique is a non-linear projection from multi-dimensional space onto two-dimensional map. The topology preserving projection is achieved via non-linear

Table 1 Structure alerts (SAs) used in the modelling

SAs Order Number in the Kohonen Map	SAs and Appropriate Mechanisms of Action	Structure of SAs	Amount of SAs in the Set
1	**SA_7: Epoxides and aziridines** Alkylating, Direct Acting Agents	 R = any atom/group	22
2	**SA_8: Aliphatic halogens** Alkylating, Direct Acting Agents	 R = any atom/group	47
3	**SA_13: Hydrazine** Alkylating, Indirect Acting Agents	 R = any atom/group	32
4	**SA_13+SA_27**	See SA_13 and SA_27	12
5	**SA_21: Alkyl and Aryl N-nitroso groups** Alkylating, Indirect Acting Agents	 R_1 = aliphatic or aromatic carbon; R_2 = any atom/group	107
6	**SA_27: Nitro-aromatic** Aminoaryl DNA Adducts Forming, Indirect Acting Agents	 Ar = any aromatic/heteroaromatic ring	75
7	**SA_27+SA_28**	See SA_27 and SA_28	14
8	**SA_28: Primary aromatic amine, hydroxyl amine and its derived esters** Aminoaryl DNA Adducts Forming, Indirect Acting Agents	 Or amine generating group Ar = any aromatic/heteroaromatic ring; R = any atom/group	52
9	**SA_X**	X- others SAs used in modeling	110
10	**NA**	No alert	334

algorithm known as training. The fundamental property of the trained network is that the similar objects are located close to each other. Therefore, it is expected that chemicals with similar structure will form the clusters. The case of our examination includes the analysis of similarities in non-congeneric set of substances using three kinds of 2D maps: the Kohonen top map (distribution of chemicals), weight levels maps (distribution of individual descriptors) and corresponding response surface output layer with distribution of carcinogenicity class (carcinogens/non carcinogens). All maps are located one under another which enable analyse and compare the obtained data.

Results and discussions

How selected descriptors correlate with SAs for carcinogenicity

The distribution of the chemical classes coded as SAs over the Kohonen map in the model for prediction of SAs

As was pointed above the eight MDL descriptors that maximally correlated with carcinogenicity were selected in the predictive model for carcinogenicity (***model_A***). The following question arises: how selected descriptors are correlated with SAs for carcinogenicity with relevant possible mechanisms of action? For this reason the CP ANN predictive model for SAs was built (***model_A_SA***). This model was aimed to get insight into the distribution of chemical classes coded as SAs over the Kohonen map.

Models for prediction of SAs were created using the same parameters like in the case of predictive models for carcinogenicity as was described in the article [8]. We examined model (***model_A_SA***) based on eight MDL descriptors, with 35 × 35 dimensional ANN trained for 800 epochs.

The chemicals containing the following SAs were considered in this study: *SA_7*- epoxides and aziridines (22 chemicals), *SA_8*- aliphatic halogens (47 chemicals), *SA_13*- hydrazine (32 chemicals), *SA_21*- alkyl and aryl *N*-nitroso groups (107 chemicals), *SA_27*- Nitro-aromatic amine (75 chemicals) and *SA_28*- primary aromatic amine, hydroxyl amine and its derived esters (52 chemicals). We also considered chemicals containing two SAs in one molecule: *SA_13* + *SA_27* (12 chemicals) and *SA27* + *SA_28* (14 chemicals). The studied data set contains the 334 chemicals marked as *NA*-no alert. These chemicals don't contain the carcinogenic SA as was determined in Toxtree. Others alerts were marked as a *SA_X*.

Table 1 represents the SAs used in the modelling with an appropriate mechanism of action, the structure of a special SA, and total number of chemicals containing particular SA in the dataset. The chemical classes coded as SAs are numbered (**1**–**10**). They are also shown in the Kohonen map (see Fig. 1). The Kohonen map enables to get insight within congeneric sets of chemicals and to determine the similarities or dissimilarities within groups of chemicals characterised on the basis of particular carcinogenic SA.

Figure 1(a, b, c, d, e) shows the weight levels maps of particular SAs and NA. The following groups of chemicals generate clusters: **5**-*SA_21* (nitro compounds) (Fig. 1a), **7**-*SA_27* + *SA_28* (nitro-aromatic, primary aromatic amines) (Fig. 1b) while others groups of chemicals marked as **1**, **3**, **4**, **6**, **9** and **10** scatter in the whole map. The following groups of chemicals generate clusters and spread throughout the map: **8**-*SA_28* (primary aromatic amines) (Fig. 1c) and **2**-*SA_8* (aliphatic halogens) (Fig. 1d). In the following part of study we considered the possible mechanism of action for chemicals containing the particular SA and tried to find reasons of clustering or scattering of studied chemicals over the Kohonen map.

Thus, the first group of chemicals (*N*-Nitroso compounds containing *SA_21*) marked as (**5**) generate the cluster. The chemicals in this group belong to alkylating, indirect acting agents as was described in papers [11, 13]. The *N*-nitrosamines and *N*-nitrosamides represent a well established class of chemical carcinogens [11]. The second group of chemicals (the nitro-aromatic, primary aromatic amines containing *SA_27* + *SA_28*) marked as (**7**) also forms the cluster. The chemicals in this group belong to aminoaryl DNA-adducts forming, indirect acting agents [11]. The clusters of chemicals containing those two alerts (*SA27* + *SA28*) are placed in the closest neurons because of similarity due to presence of the same groups responsible for the similar mode of action or biological activity. It is interesting that all chemicals in this cluster are also positive by results of mutagenicity tests (Salmonella typhimurium TA98 strain).

The chemicals coded as *SA27*, *SA28* and groups of chemicals containing (*SA27* + *SA28*) and their structures are shown in Tables 2SI–4SI in supplementary materials "Online resource". Why chemicals containing *SA27* (**6**) and *SA28* (**8**) spread throughout the Kohonen map while group of chemical containing two SAs (*SA27* + *SA28*) (**7**) forms a cluster? Analysing structures of chemicals with *SA_27* (75 chemicals) and *SA_28* (52 chemicals) one can notice greater diversity of represented structures here in comparison with group of chemicals containing *SA27* + *SA28* (14 chemicals). Moreover, the studied chemicals in the dataset contain not only *SA 27* or *SA 28* but others functional groups which effect the activity of chemicals. The MDL descriptors possess such features that enable to discriminate between chemicals with great structural diversity. The SA approach in some cases unable to make differences inside group of chemicals with great diversity of structure because it

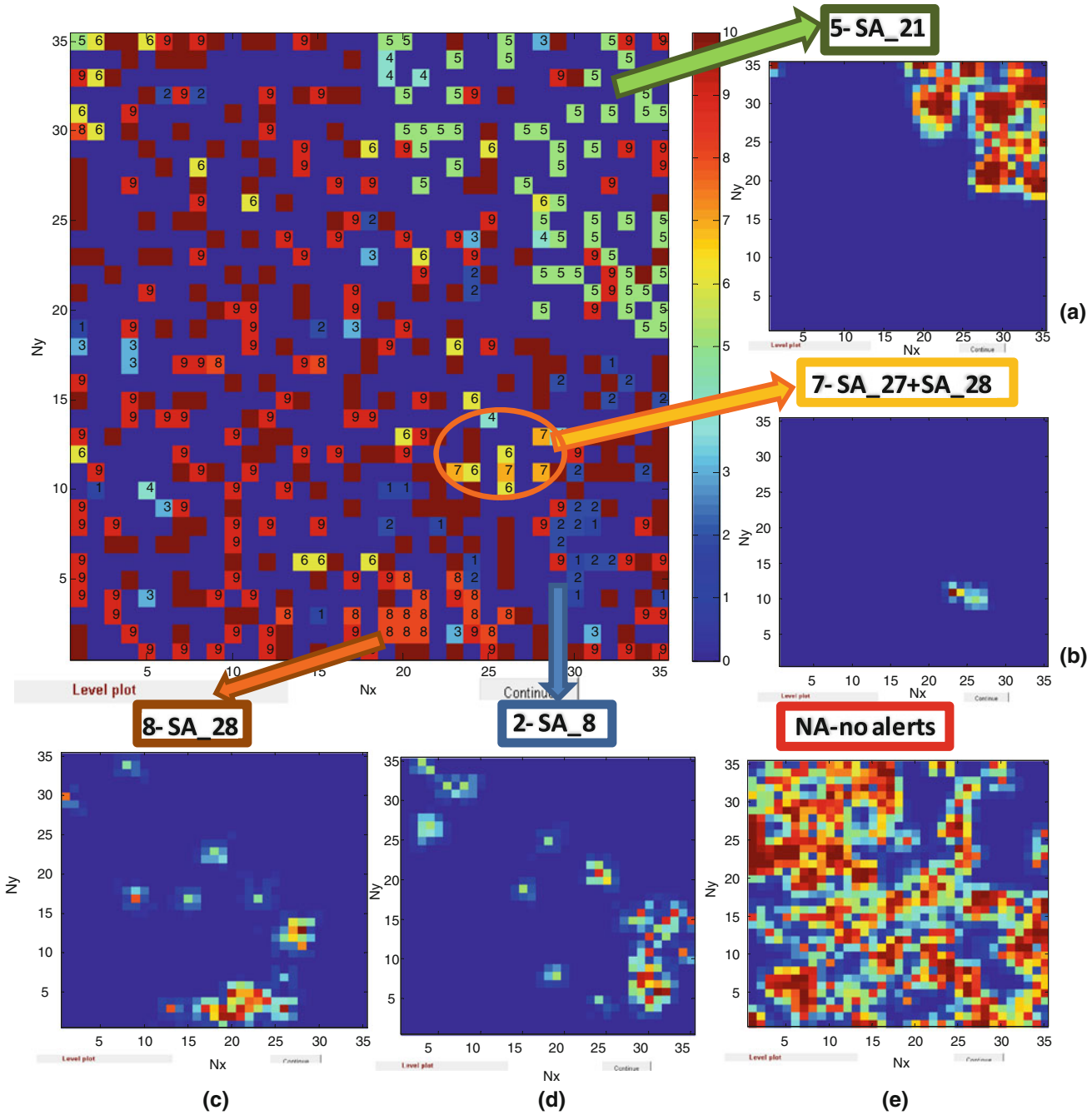


Fig. 1 The top map of ***model_A_SA*** for prediction of SAs illustrating the distribution of largest selected families of chemicals containing appropriate SAs (from **1** to **9***) complemented with the following weight maps demonstrating: **a** The distribution of chemicals containing *SA_21*- (**5**); **b** The distribution of chemicals containing (*SA_27* + *SA_28*)-(**7**); **c** The distribution of chemicals containing *SA_28*- (**8**); **d** The distribution of chemicals containing *SA_8*- (**2**); **e** The distribution of chemicals containing *NA* (no alerts) *The numbers (**1**–**9**) in the top map relate to chemicals containing the following SAs: **1**-*SA7*, **2**-*SA8*, **3**-*SA13*, **4**-(*SA13* + *SA27*), **5**-*SA21*, **6**-*SA27*, **7**-(*SA27* + *SA28*), **8**-*SA28*, **9**-*SA_X* (X-others SA)

doesn't take into account presence of others (non-carcinogenic) functional groups which affect activity of compounds.

The next step we considered the third group of chemicals marked as (**2**) (*Aliphatic halogens* containing *SA_8*) (see Fig. 1d) which forms small clusters and are spread throughout the map. This phenomenon can be explained the following way. As was described in the paper [7], "…the action mechanisms of aliphatic halogens tend to shift from genotoxic to epigenetic, with increasing degree of halogenation and depending on the carbon skeleton

(linear chains or cyclic structures). Short-chain monohalogenated alkanes (and alkenes) are potential direct-acting akylating agents; dihalogenated alkanes are also potential alkylating or cross-linking agents (either directly or after GSH conjugation). Polyhaloalkanes act by free radical or nongenotoxic mechanisms, or may undergo reductive dehalogenation to yield haloalkenes. For what concerns halogenated cycloalkanes (and cycloalkenes), the mechanism of carcinogenic action is unclear…" We suppose that chemicals from this family that have similar mechanism of action are placed in the cluster. Others chemicals that have different mechanism of action described above are scattered in the map. This fact needs more deep investigations.

As for distribution of chemicals marked as *NA* (no alert) (334 chemicals) one can see that chemicals are disperse widely in the map (see weight level map of *NA* in the Fig. 1e). Prediction of non-carcinogens per se represents challenge in some approaches. Thus, in the case of regression models [3] for congeneric series of chemicals in some cases good prediction results were reported for carcinogenic potency, while the equations (for instance, in the case of aromatic amines) did not predict well the absence of carcinogenic effect for non-carcinogens. Thus, differentiating between active and inactive compounds (yes/no activity) and determination of carcinogenic potency was done using different descriptors within the same class of chemicals [3]. The CP ANN model for prediction carcinogenicity, in turn, enable to predict 75% of carcinogens (SE-sensitivity) and 69% of non-carcinogens (SP-specificity) [8] using the same descriptors.

The correlation between descriptors and nitroso compounds in the model for prediction SAs

The second goal of our research was to find out if correlation exists between some families of chemicals used in the data set and descriptors applied in the models.

The cluster of nitroso compounds (the population of chemicals marked as (**5**) related to *N*-Nitroso compounds containing *SA_21* [alkyl and aryl *N*-nitroso groups (107 chemicals)] was found in the right top corner in the top map of the ***model A_SA*** (Fig. 2a).

Figure 2b illustrates the cluster of chemicals containing *SA_21* while Fig. 2c demonstrates the influential zone of MDL descriptor **D3** (*SdsN_acnt* -Count of all (=N) groups in molecule). The overlapped areas prove that the MDL descriptor **D3** is descriptive for the structural features intrinsic for nitro compounds containing *SA_21* (alkyl and aryl *N*-nitroso groups).

It should be noted that no clear visible clusters demonstrating correlation between applied descriptors and other selected families of studied chemicals were found.

Thus, the results of supervised learning show that the descriptor space used in this study preserves visible information about molecular similarities in terms of SAs for nitrosocompounds.

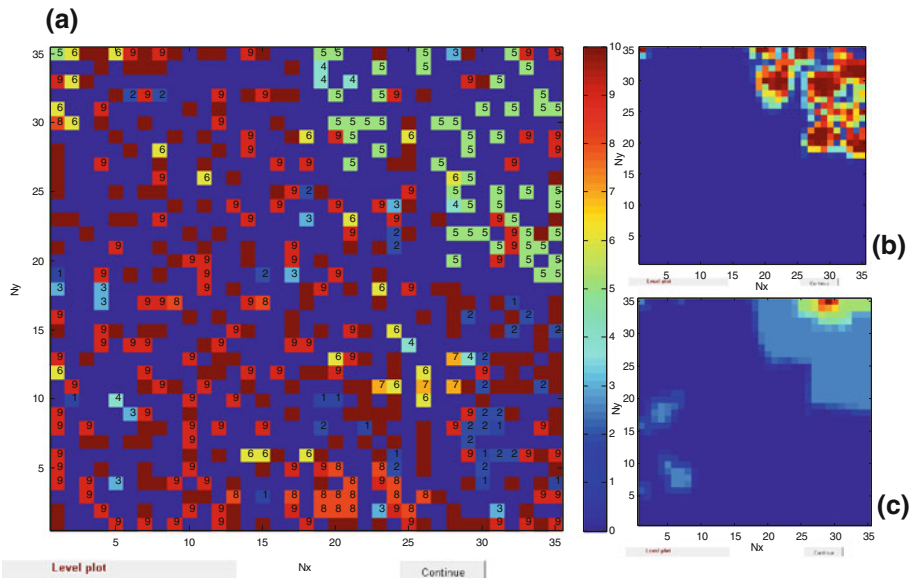


Fig. 2 **a** The top map of ***model_A_SA*** for prediction of SAs illustrating the distribution of largest selected families of chemicals containing appropriate SAs (from **1** to **9***) complemented with the following weight maps demonstrating: **b** the distribution of chemicals containing *SA_21* (**5**); **c** the distribution of values of MDL descriptor **D3** (*SdsN_acntnt*- Count of all (=N) groups in molecule). *The numbers (**1**–**9**) in top map relate to chemicals containing the following SAs: **1**-*SA7*, **2**-*SA8*, **3**-*SA13*, **4**-(*SA13* + *SA27*), **5**-*SA21*, **6**-*SA27*, **7**-(*SA27* + *SA28*), **8**-*SA28*, **9**-*SA_X* (X-others SA)

Mechanistic interpretation of the CP ANN predictive model for carcinogenicity

The features of CP ANN predictive model for carcinogenicity

The model for prediction of SAs was discussed in the previous chapter (***Model A_SA***). In this part of the article we took into consideration the model (***Model A***) for prediction a carcinogenic class [carcinogen (**2**) or non-carcinogen (**1**)] and examined the relationship between the carcinogenic potency, the structure of chemicals and the applied descriptors. The input variables in the model for prediction of SAs and carcinogenic potency were the same (numeric representation of the same descriptors).

The carcinogenic property of the compounds is related to the molecular structure in a complex way due to the diversity of the molecules in the studied dataset. The descriptors used in the study encode different aspects of the molecular structure. We have used the CP ANN and combined the mapping capability of a Kohonen network with a supervised learning strategy. The focus of our investigations was the analysis of pattern levels in the weights of trained network which provide the researcher with a deeper knowledge about mechanistic background related to the effect of individual variables visualized and estimated from the formed clusters.

As was mentioned above the first attempts for mechanistic interpretation of model for prediction of carcinogenicity class using 8 MDL descriptors (***model A***) were made in the article [8]. The present paper resumes and upgrades the interpretation of models represented in the previous study. We have used Kohonen maps to show connection between the descriptors, the structure features of chemicals fell in the most influential zones of individual descriptors and the corresponding carcinogenic potency.

The correlation between descriptors and nitroso compounds in the predictive model for carcinogenicity

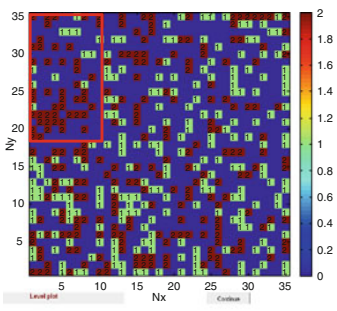
Figure 3 presents the top map of ***model A*** with distribution of carcinogens (**2**) and non-carcinogens (**1**) (Fig. 3b), the weight map of MDL descriptor **D3** (Fig. 3c) and marked with rectangle in Fig. 3b and c fragment of the upper left section of the top map of ***model A*** (Fig. 3a).

The area located in the left upper corner of the top map in Fig. 3b marked with rectangle is populated with majority of chemicals carcinogens (**2**). This area marked with rectangle corresponds to fragment shown in Fig. 3a with coordinates (n_x from 1 to 10 and n_y from 19 to 35) labelled with ID of chemicals occupying individual neurons with indication of carcinogenic SAs. Additionally, the weight map of MDL descriptor **D3** (*SdsN_acnt* -count of all (=N) groups in molecule) is shown in Fig. 3c. The majority of chemicals located in selected area (rectangle) contains the *SA21*. Others compounds contain the following SAs: *SA9*, *SA 13*, *SA14*, *SA27*. Thus, Fig. 3 demonstrates the relationship between the MDL descriptor **D3** (*SdsN_acnt -count of all*

(a)

35	46, 256, 262, 447 SA28,28bis,29,15		448, 640 SA21,25, 22	280 SA27			800 NA	191 SA13		114, 401, 672 SA21, NA
34			562 SA21							452 SA21
33					263 SA21	64 NA	68 SA22			
32		565, 610 SA21								619 NA
31	584, 603, 729 SA21	457 SA21		546 SA21		539 SA21				
30								589 SA21	605 SA21	
29	581, 582 SA21,25		604 SA21,26		472, 586 SA21		473 SA21			
28	90, 598, 599, 600, 601, 636 SA15, 21						455 SA21			
27				554, 555 SA21,31a, 27					574 SA21	
26	430 NA						597 SA21	5, 501 SA27,13		
25	249, 563 SA21		569 SA21		474 SA21			560 SA21		
24	17, 205 NA, SA21				70, 319 SA14,21		588 SA21	383 SA13,27		
23	2 NA			587 SA21	305 SA14					
22	580 SA21	188 NA	579 SA21	400 SA9		578 SA21	551, 573 SA21	543,545, 594 SA21		
21		583 SA21	609 SA21	71 SA14	595, 596 SA21					
20	558 SA21		577, 590 SA21		72 SA14			566, 567 SA21		
19	557, 585 SA21	553, 602 SA21								
nx ny	1	2	3	4	5	6	7	8	9	10

(b)



(c)

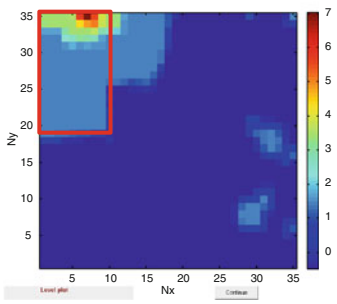


Fig. 3 **a** A fragment of the upper left section of the top map 35 × 35 of ***model A*** for prediction of carcinogenicity with coordinates (n_x from 1 to 10 and n_y from 19 to 35) labelled with ID of chemicals occupying individual neurons with indication of SA; **b** A top map of ***model A*** with distribution of carcinogens (**2**) and non-carcinogens (**1**); **c** A weight map of MDL descriptor **D3** (*SdsN_acntnt*- Count of all (=N) groups in molecule). Fragment (**a**) corresponds to clusters marked as red rectangle in (**b**) and (**c**)

(=*N*) *groups in molecule*) and chemicals containing *SA21* (*alkyl and aryl N-nitroso groups*), *SA9* (*Alkyl nitrite*), *SA 13* (*Hydrazine*), *SA14* (*Aliphatic azo and azoxy*) and *SA27* (*Nitro-aromatic*).

Descriptors identify the certain structural features or particularities. Thus, we have found the relationship between descriptors containing features for nitro compounds that gave ability to neural network to organize those families of chemicals in topologically near locations (neurons). The majority of chemicals from this class are carcinogens, i.e. possess the same biological activity. Obviously, the nitro SAs are important for carcinogenic activity which is in good agreement with the selection of MDL descriptor **D3** that resulted from our modelling methodology.

The study of the influential zones of descriptors using their weight maps

The following part of our study was dedicated to research of influential zones of descriptors (areas with the largest values) and there correlation with structure of chemicals located in those areas. Analysing the individual descriptors layers (weight maps) in the Self-Organizing Maps (Kohonen map) one recognized the importance and role of individual descriptors in a studied model. The results of our investigations represented in the Supplementary material Tables 1SI–6SI of the Online Resources.

We have found that influential zones of some of descriptors like MDL descriptors **D1** (*SdsCH*- Sum of all (=CH–) E-State values in molecule) (Fig. 1SI), **D2** (*SdssC_acnt*- Count of all (=C<) groups in molecule) (Fig. 2SI), **D4** (*dxp9* -Difference simple 9th order path chi indices) and **D7** (*SHCsats*- sum of hydrogen E-State on sp3 C on saturated bond) (Fig. 4SI) cover only small area. It was found that the pointed descriptors have the highest value only for few chemicals in the studied dataset. In contrast, the MDL descriptor **D6** (*Gmin- Smallest atom E-State value in molecule*) (Fig. 5SI) has influential zone which covers the whole map. This phenomenon probably is the evidence that descriptor **D6** has features effecting on majority of chemicals in the dataset.

The MDL descriptor **D3** (*SdsN_acntnt*- Count of all (=N) groups in molecule) (Fig. 3SI) forms the cluster related to nitroso compounds with *SA21* (alkyl and aryl *N*-nitroso groups) distributed at the same location in Kohonen maps as pointed descriptors. The majority of these chemicals are carcinogens here.

The more detailed characterization of MDL descriptors used in the modeling and their mechanistic interpretation is given in supplementary materials of the Online Resource (see Table 1SI and Figs. 1SI–6SI).

Descriptors used in the QSAR models as a general features relevant to carcinogenicity

The goal of a QSAR approach is to find out general features relevant to carcinogenicity. The question arises: is it possible to find out descriptors used in QSAR models for prediction of carcinogenicity that contain features relevant to carcinogenicity. In contrast to investigations related to congeneric chemicals, the so-called "general" studies for non-congeneric chemicals contributes for searching of general features related to carcinogenicity and relevant to different classes of chemicals.

It is well known that mutagenicity and genotoxicity related to each other and some of genotoxic carcinogens can be also mutagenic. We have considered some models for prediction of mutagenicity, genotoxicity and carcinogenicity to find out if different QSAR approaches for prediction endpoints listed above have similar or the same features related to mutagenicity, genotoxicity or carcinogenicity. The following descriptors that were selected in our models for prediction of carcinogenicity [8] were found in the models created by Votano [14] and Contrera [15]:

SdsCH Sum of all (=CH–) E-State values in molecule [8, 15];
SdssC_acnt Count of all (=C<) groups in molecule [8, 15];
SdsN_acnt Count of all (=N) groups in molecule [8, 14, 15];
Gmin Smallest atom E-State value in molecule [8, 14, 15];

SHBint2_Acnt Count of internal hydrogen bonds with 2 skeletal bonds between donor and acceptor [8, 15].

As was shown above, some of the E-state descriptors relate directly to known sturctural alerts for carcinogenicity [for example, *SA21* (*alkyl and aryl N-nitroso groups*)]. Each of E-state descriptors encode the following three aspects of structure: electron accessibility as well as presence/absence and the count of the atom or bond.

A model based on E-state descriptors represented as continues values can correlate with carcinogenicity to a specific range of descriptor value, whereas the use of SAs limits the model to the presence or absence of a given fragment (SA). The consideration of a SA fragment per se does not take into account steric and electronic surrounding in the whole molecule that can deminish or enhance carcinogenic potency. This can render the fragment non toxic, or create a toxic fragment that has not previously been identified. From other hand, SA approach provides the clear determination of mechanism of action for particular SAs that can be successfully used in a mechanistic interpretation of QSAR models. We belive that an integration of

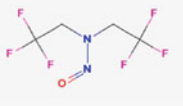
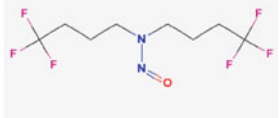
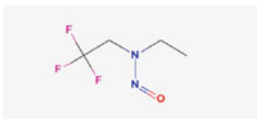
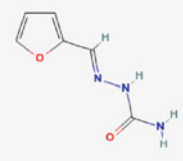
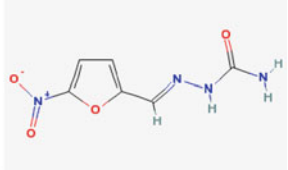
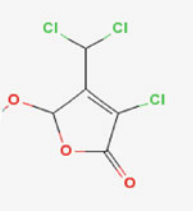
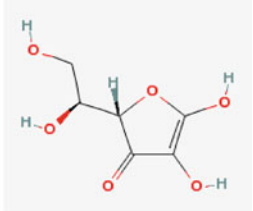
different approaches and tools contributes into larger perspective of the risk assesment process.

How energy of activation ΔE in processes of metabolism affect carcinogenic potency of chemicals with similar structure

As a rule, the chemicals located in the same neuron of the Kohonen map in the training and test set have the similar structure. In some cases the activity of chemicals with the similar structure can differ resulting in a false prediction. We have demonstrated here three examples of false prediction due to the different energy of activation ΔE in the processes of metabolism.

Firstly, we considered the biologically inactive chemical *N-Nitrosobis(2,2,2-trifluoroethyl) amine* (CASRN 625-89-8) which is a non-carcinogen. This chemical was predicted as carcinogen (FP- false predicted) because the chemical with similar structure *N-Nitroso-bis-(4,4,4-trifluoro-N-butyl)amine* (CASRN 83335-32-4) from the training set located in the same neuron is carcinogen (see Table 2). This phenomenon was described in the papers [16, 17] dedicated to investigation of a bioactivation of compounds in the process of a metabolic biotransformations using the oxenoid model and the quantum chemical calculation. The authors explained how the carcinogenic activity depends on the value of activation energy ΔE in the oxidation reaction. The bioactivation of *N-Nitrosobis-(2,2,2-trifluoroethyl) amine* is not possible because the energy

Table 2 Structures of false predicted chemicals from test and training set located in the same or closest neurons

No	Position of neurons n_x*n_y in test/training set	The chemical from the test set	The chemical from the training set	SA	FP or FN
1	35x35/35x35	 N-Nitrosobis(2,2,2-trifluoroethyl) amine; (CASRN 625-89-8); **non-carcinogen**; ΔE= 27.21 kcal/mol	 N-Nitroso-bis-(4,4,4-trifluoro-N-butyl)amine; (CASRN 83335-32-4); **carcinogen**; TD50=0,745 mg/kg; ΔE= 22.92 kcal/mol  N-Nitroso(2,2,2-trifluoroethyl) ethylamine (82018-90-4); **carcinogen**; TD50=2,52 mg/kg; ΔE= 23.42 kcal/mol	SA21	FP
2	17x33/17x33	 2-Furaldehyde semicarbazone; (CASRN 2411-74-7); **non-carcinogen**	 Nitrofurazone; (CASRN 59-87-0); **carcinogen**	SA13	FP
3	27x26/28x26	 3-Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone(MX); (CASRN 77439-76-0); **carcinogen**	 L-Ascorbic acid ; (CASRN 50-81-7); **non-carcinogen**	SA8	FN

* *FP* false positive, *FN* false negative; SA8-Aliphatic halogens; SA13-Hydrazine; SA21-alkyl and aryl *N*-nitroso groups, ΔE-energy of activation

of activation is too high (ΔE=27.21 kcal/mol). Only chemicals with the energy of activation lower then 27 kcal/mol possess the carcingenic potency. In the case of the *N-Nitroso-bis-(4,4,4-trifluoro-N-butyl)amine* the energy of activation (ΔE) is lower and equal to 22.92 kcal/mol therefore this compound is carcinogen.

Secondly, we studied the *2-Furaldehyde semicarbazone* (CASRN 2411-74-7) which is a non-carcinogen (see Table 2). The chemical in the training set located in the same neuron *Nitrofurazone* (CASRN 59-87-0) is a carcinogen. According to analysis presented in article [17] *Nitrofurazone* (CASRN 59-87-0) has the nitro group as a substitute in the furan ring. It was found that in the course of metabolism the nitrogroup of this compound is restored to a hydroxylamine metabolite, then nirenium ions and cation–radicals. Then the active forms of oxygen are formed, which results in the carcinogenic potency of studied chemical. The bioactivation of *2-Furaldehyde semicarbazone* in turn is not possible (absence of the nitro group as a substitute in the furan ring). Therefore *2-Furaldehyde semicarbazone* was predicted as carcinogen (FP- false positive).

Finally, we explored compound *Chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone(MX)* (*CAS 77439-76-0*) (see Table 2) which was predicted as false negative (FN). (This chemical we marked as MX). It is known that chlorine atoms result in the mutagenic and carcinogenic properties of MX. This chemical has genotoxic alert SA8 (Aliphatic halogens) according to Toxtree [7]. MX is a direct carcinogen able to form adducts with DNA, in particular, as a result of reaction with the $CHCl_2$ group. In training set the *L-Ascorbic acid* (CAS 50-81-7) (non-carcinogen) with similar structure placed in closed neuron. This chemical is no-carcinogen because it doesn't contain atom of halogen in the structure.

Summarising analysis given above we can conclude that using the knowledge about the bioactivation of chemicals in the case of indirect chemicals carcinogens can bring the explanation why the chemical with similar structure can have in some cases different biological activity.

Conclusion

The new models for prediction of the carcinogenicity for regulatory purpose using CP ANN were proposed recently [8]. The descriptors employed in the modelling contain information about topological characteristics, polarizability and charge distribution of molecules related to the reactivity but don't include information about a carcinogenic mechanism of action which is important in a mechanistic interpretation of models. Therefore, the knowledge-based Toxtree approach based on carcinogenic SAs (containing information about a carcinogenic mechanism of action) was integreated with the statistically-based method (CP ANN) to obtaine the mechanistic interpretation of models.

The mechanistic insight in the CP ANN model was demonstrated using the inherent mapping technique (i.e. Kohonen maps) which enables the visualization of the following features in 2D space: the carcinogenic potency; the distribution of descriptors in individual layers which express the structural and electronic features related to activity of molecules as well as the distribution of groups of chemicals containing the specific carcinogenic SAs related to a mechanism of action.

In this study we demonstrated that the MDL descriptors possess such features that enable to discriminate between chemicals with great structural diversity. The advantage of the CP ANN model is in the ability to obtain a non-linear topological distribution of several small clusters of particular chemicals that are based on different modes of action. The SA approach in turn is limited to make differences inside group of chemicals with great diversity of structure because it is oriented only on SAs.

It was shown that some E-state descriptors like the MDL descriptor **D3** (*SdsN_acntnt*- Count of all (=N) groups in molecule) relate directly or are associated with known SAs (like *SA21* [alkyl and aryl *N*-nitroso groups)] for carcinogenicity for such classes of chemicals like nitro compounds, nitro-aromatic, primary aromatic amines, and consequently carcinogens and non-carcinogens.

We demonstrated a few descriptors used in QSAR modelling by different researchers (Voltano, Contrera) containing the general features relevant to carcinogenicity.

The results presented in the articles demonstrate the transparency of CP ANN algorithm which is one of the main features of QSAR models used for regulatory purposes. The mechanistic interpretation of models is very important for assessment of safety of chemicals in the risk assessment.

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