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Subchronic Oral and Inhalation Toxicities: a Challenging Attempt for Modeling and Prediction

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Abstract: The article deals with a challenging attempt to model and predict "difficult" properties as long-term subchronic oral and inhalation toxicities (90 days) using nonlinear QSAR approach. This investigation is one of the first to tackle such multicomplex properties where we have employed nonlinear models based on artificial neural network for the prediction of NOAEL (no observable adverse effect level). Despite the complex nature of the NOAEL property based on in vivo rat experiments, the successful models can be used as alternative tools to non-animal tests for the initial assessment of these chronic toxicities. The model for oral subchronic toxicity is able to describe 88%, and the inhalation model 87% of the statistical variance. For the sake of future predictions, we have also defined in a quantitative way the applicability domain of all neural network models.

Keywords: Subchronic oral toxicity · Subchronic inhalation toxicity · Artificial neural network · QSAR · NOAEL

1 Introduction

Chronic toxicity is a consequence of the persistent or progressively deteriorating dysfunction of cells, organs or multiple organ systems, resulting from long-term exposure to a chemical. Of relevance for cosmetic products are the oral, dermal and inhalation subacute (28 days) and subchronic (90 days) repeated dose studies in rodents. The 28-day or 90-day oral toxicity tests in rodents are the most commonly used long-term toxicity tests. The highest dose administered is designed to cause some toxicity, but not lethality.

In the notification process of dangerous substances, long-term toxicity studies are required when the substance under consideration is produced or imported in amounts exceeding 1 ton/year.[1] In the case of the development of cosmetic ingredients that have specific biological properties and which will come into contact with human skin for a long period of time, evaluation of the systemic risk is a key element in evaluating the safety of these new ingredients, irrespective of the tonnage-linked and possibly restricted requirements imposed by the Dangerous Substances Directive.[2] Therefore, in certain cases the use of animal long-term experiments to study one or more potential toxic effects remains to be required by law. However, it is essential that the aim of abolishing animal experiments for testing cosmetic products be pursued and that the prohibition of such experiments becomes effective in the territory of the EU Member State. The 7th Amendment to the Cosmetic Directive 76/768/EEC allows 10 years from the entry into force date on, to come up with validated alternative tests for repeated exposure.[3] Currently, one of the alternative methods which are scientifically adopted/validated by the Organization for Economic Cooperation and Development[4,5] and applicable to the large part of the chemical sector is Quantitative-Structure Property/Activity Relationship (QSPR/QSAR).[6] One of the main advantages of this modeling technique is that it does not require direct laboratory experiments on animals but rather already available data on such experiments. A developed QSAR model can be used to predict untested chemicals for their subchronic toxicity without the need of new animal tests.

According to OECD guideline 413[7] the subchronic inhalation toxicity studies are primarily used to derive regulatory concentrations for assessing worker risk in occupation settings. They are also used to assess human residential, transportation, and environmental risk. Such a guideline would enable the characterization of adverse effects follow-

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ing repeated daily inhalation exposure to a test article for 90 days.

A fast growing and modern area nowadays is the nanoparticle science.[8] It finds applications in various technologies related to bio, medicinal, eco, and toxicity areas. Therefore the assessment of the subchronic inhalation toxicity is important factor for the evaluation of the human exposure. For instance, the experimental evaluations of the gold nanoparticles approximated using rats have been carried out by Sung et al.[9] The author concluded that the lungs were the only organ in which there were dose-related changes in both male and female rats. Changes observed in lung histopathology and function in high-dose animals indicate that the highest concentration (20 $\mu g/m^3$) is a lowest observable adverse effect level (LOAEL) and the middle concentration (0.38 $\mu g/m^3$) is a no observable adverse effect level (NOAEL) for this study. Similar study was also done by the same author on silver nanoparticles.[10] The results indicated dose-dependent increases in lesions related to silver nanoparticle exposure, including mixed inflammatory cell infiltrate, chronic alveolar inflammation, and small granulomatous lesions. Target organs for the silver particles were considered to be lungs and liver in the male and female rats.

Other important area where humans can be exposed to toxic inhalation environment is the area where volatile organic compounds (VOCs) are involved. Large part of the hydrocarbons, including nitrogenous, chlorinated and sulfurated organics can be classified as VOCs. Often these compounds are used in technologies as manufacturing or development of solvents, e.g. petrochemical, pulp, coating or hard resin industries. Kim et al. have examined the subchronic inhalation toxicity (LOEAL, NOEAL) of 1,3-dichloro-2-propanol VOCs,[11] dimethyl disulfide.[12] The author performed analysis on clinical signs and mortality, body weight changes and food consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry etc using rats in order to define the adverse effects. The target organs investigated were lung, liver, kidney and blood vessels in rats. The results concluded that the adverse effects are dose-dependent characteristics.

The second aspect discussed in this work is related to subchronic oral toxicity in rodents. The repeated dose 90-day oral toxicity was determined using the OECD test guideline 408 (EU B.26).[7] This test guideline has been designed to fully characterize test article toxicity by the oral route for a subchronic duration (90 days). It can provide an estimate of a no-observed-adverse-effect level (NOAEL) of exposure which can be used in selecting dose levels for chronic studies and for establishing safety criteria for human exposure. The importance of the toxicity levels (if any) for newly developed dietary products is enormous. There are numerous works addressed to the experimental assessment of subchronic oral toxicity using animals (mainly rats) and related to various chemicals such as aloesin,[13] CP-31398,[14] resveratrol,[15] D-psicose,[16] L-serine,[17] monatin salt.[18]

Currently no generally accepted alternative methods are available for replacing repeated-dose in vivo testing. Complete replacement of animal usage in these areas represents an enormous scientific and technical challenge. Since a wide range of endpoints are investigated in in vivo chronic toxicity studies, an integrated approach to repeat-dose toxicity testing based on the use of alternative methods with complementary endpoints will need to be developed.

To our knowledge, there are very few scientific works published on the theoretical prediction of both, *i.e.* inhalation and oral subchronic toxicities (90 days). One of the related studies by Garcia-Domenech et al.[19] deals with the prediction of LOEAL. The authors used 87 non-homogenous compounds extracted from EPA database to develop multilinear predictive models based on topological indices and the ITS (internal test set) method. However, the authors concluded that some of the obtained models are of poor quality.

It is known that these properties depend on a large number of characteristics which make their modeling a difficult task, especially when non congeneric compounds are involved. Also, factors such as target organs (lungs, kidney, blood, hart etc), metabolic pathways, experimental setups, dosage, animals etc contribute to the complication of the prediction. Therefore, the development of alternative methods avoiding animal test is of great importance.

It should be mentioned that the experimental data collected for the current work and the definition of the NOAEL property allow interpretation of the results in the general form which regards different organ/tissue (blood, gastro intestinal tract, kidney, heart etc). Moreover, fewer data were available in the literature related to a single organ/tissue and thus were not statistically significant for a model. Therefore, analysis of the mechanism of action has to be performed in generalized sense.

The aim of our current study is to attempt to obtain predictive theoretical models for these "difficult" properties – subchronic oral and inhalation toxicity. Our approach is based on nonlinear QSAR using artificial neural networks (ANNs). Thus, the models herein can be used as alternative methods for prediction or decision making of both toxicities.

2 Methods

2.1 Oral Subchronic Toxicity Data

In this study, we proceeded from NOAEL data, which were originally expressed in units of %, ppm or milligrams of chemical per kilogram (mg/kg) and extracted from IUCLID database.[20] The database integrates the subchronic oral toxicity for 90 days of rats from of multiple sources of different authors. For the further model development, the

NOAEL values were converted to their corresponding mmol/kg units in order to standardize the value for each chemical according to its molar concentration. All calculations herein were performed on log (1/NOAEL) (or pNOAEL) in order to utilize the normal statistical distribution of data. In the selection of data points, we have tried to extract data with as similar as possible experiment positions (dosage, number of animals, measurements) and compounds with unambiguous structural connectivity. Altogether, 120 data points (noncongeneric compounds) with measured NOAEL values were collected related to experiments on rats (male/female). Further, the number of the data was reduced to 103 (see Section ANN model for subchronic inhalation toxicity and Supporting Information SI1).

2.2 Inhalation Subchronic Toxicity Data

The subchronic inhalation toxicity had been determined using the OECD test guideline 413.[21] The IUCLID Chemical Data Sheets were used to extract the rat subchronic inhalation NOAELs.[20] Therein, any study utilizing rats conducted for 90 days (3 months, 13 weeks) was considered a subchronic study. When several NOAELs of a chemical were available in IUCLID Chemical Data Sheets Information System, the value of the latest study was preferred. The NOAEL data originally expressed in units of ppm or milligrams of chemical per liter (mg/L). These values were converted to their corresponding mmol/m^3 in order to make the respective correlations comparable. The modeling calculations were performed on pNOAEL as dependent variable. The same considerations for the experimental data point's selection as for the oral toxicity were also taken into account here. Thus, it was extracted 71 data points (noncongeneric compounds) that were used in our ANN models (see Supplementary Information SI2).

2.3 Optimizations and Descriptor Generations

In the current study, we developed nonlinear QSAR (Quantitative Structure-Activity Relationship) models based on artificial neural networks (ANNs) for pNOAEL of both subchronic toxicities i.e. oral and inhalation. These models are based on theoretical descriptors, which were calculated solely from the molecular structure using FQSARModel program.[22] The descriptors used can be classified as: (i) constitutional, (ii) geometrical, (iii) topological, (iv) charge-related, (v) quantum chemical, and (vi) thermodynamic.[23] The total number of descriptors ranged between 600 and 900 for each compound. The structure of compounds was optimized as reflecting a random vacuum conformer with the minimum potential energy using molecular mechanics MM+[24] and then followed by MOPAC 6.0.[25] Thus, the quantum-mechanical semi-empirical calculations based on AM1[26] energy minimization was applied for the final geometries with a gradient 0.05 kcal/Å as a stopping criterion.

2.4 Artificial Neural Networks

Artificial neural networks (ANN) have been applied in many diverse scientific endeavors, ranging from economics, engineering, physics, and chemistry to medical science.[27] ANNs have been useful tools in QSAR/QSPR studies, and particularly in cases where it is difficult to specify an exact mathematical model for describing a given structure-property relationship.[28] The wide applicability of ANNs stems from their flexibility and ability to model non-linear systems without prior knowledge of an empirical model.

In our study, a fully connected neural network with back-propagation of the error[29] was constructed and used in the building of the nonlinear models for all properties. The training of the net (optimization of the weights) was performed by either standard delta rule or/and Levenberg–Marquardt algorithm.[30] The activation function used in the ANNs for the neurons was hyperbolic function.

In order to find the most important descriptors as inputs to the net, a sensitivity analysis was performed on a preselected descriptor space, based on the lowest root-mean squared error (*RMS*). This space was formed after applying the following criteria for reduction of the total descriptor space: i) all descriptors with variance less than 10^{-4} were excluded, ii) descriptors which did not indicated Pearson correlation coefficient $R > 0.2$ with respect to the property iii) by inspection of certain chemically irrelevant descriptors. Further, all the remaining descriptors were correlated with the property in order to extract (and use as inputs to the net) the best few with highest correlation coefficient. Prior to this procedure the descriptors were normalized according to their variation (distance between minimum and maximum values) and standard deviation. The main reason for such selection is that the descriptors can be explained/related to the mechanistic picture behind the property interaction in a way similar to the multilinear regression models. For instance, a positive correlation would suggest that with the increase of the descriptor value the property value would also increase.

In the search for an optimal ANN architecture the lowest possible number of neurons was sought for, in order to follow the common principle of generality of the ANN prediction.[31] Several ANN models with different architectures were built for each property. In addition, we monitored the *RMS* (or Pearson's correlation coefficient *R*) for each different architecture (regarding the hidden units in the hidden layer). This procedure was done in order to select the topology with the lowest *RMS*. The number of layers was chosen to be three- to four-fold based on the common practice for the QSAR ANN modeling and by taking into account the number of data points so that to reduce the chance for overfitting during the training stage. The whole ANN training procedures were performed by Statistica 7 software.[32]

The validation of the ANN models was carried out by using training, selection (validation) and test set. All those

subsets were constructed as reflecting the distribution of the experimental property values of the whole data set. The validation and the test sets included 1/5 to 1/3 of the total data points. The selection set is used to train the network so that to avoid overfitting by stopping the training procedure prematurely when the RMS_{sel} starts to increase. The test set can be considered as an external set in order to check the predictivity of the ANN after training.

It is important for a predictive QSAR model that a certain limits are to be defined for the future predictions of compounds i.e. applicability domain (AD). We define the applicability domain of the general ANN model quantitatively proceeding from the minimum and maximum descriptor values for the training set. Our practice showed that predictions of new compounds have to be bound within the descriptor interval $[D_{imin},D_{imax}]$ augmented by $\pm\,|D_{imax}-D_{imin}|\times 0.3$, where D_{imin}, D_{imax} are the minimum and maximum descriptor values for the training set for the *i*-th descriptor (shown in squared brackets above). This condition has to be simultaneously fulfilled for all *i* descriptors so that the ANN model would give realistic predictions.

3 Results and Discussion

3.1 General ANN Model for Oral Toxicity Based on the Total Number of Compounds. Improved ANN Model Without S-Containing Compounds

The first attempt to model the oral toxicity was based on the total set of 120 chemicals. The initial descriptor pool for this property consisted of approximately 900 descriptors per structure. All descriptors were correlated with the property (pNOAEL) and then the first 20 descriptors (preselected from the refined pool as described in Section 2, Methods) were selected that indicated the largest correlation coefficient. Next, 11 networks with different structures and inputs from the preselected 20 descriptors were tested in order to find the best ANN with lowest *RMS* (root-mean-squared error) and highest *R* for training, selection and test sets. The best ANN model that was found had 8 inputs. After that, 107 epochs were used to train the final network with architecture depicted as 8-7-6-1 (8 neurons in the input layer, 7 neurons in the first hidden layer, 6 neurons in the second hidden layer and one neuron for the property in the output layer). Optimization of the weights was performed with Levenberg–Marquardt algorithm[30] using linear (inputs) and hyperbolic (hidden) and logistic (output) activation functions. In this part of the modeling, all data were divided by random manner following the property distribution into training, selection (validation) and test (external) sets with 90, 15 and 15 data points, respectively.

The best model obtained after training showed satisfactory statistics. The correlation coefficient *R* (and *RMS*) for the training, selection and test sets were 0.781 (0.129), 0.639(0.185) and 0.731(0.119) respectively (see Table 1). The linear plots between the predicted and observed property are shown in Figure 1. In the plot are also indicated the prediction bands (dashed lines) at 95% confidence level.

The descriptors in this model were selected by their *R* as described in Materials and Methods. The eight descriptors found to be significant for the ANN were *LogP*, *Kier&Hall index (order 3)*, *Number of halogenated groups*, *Highest total interaction (AM1) for O–H bonds*, *Average atom weight*, *Hydrogen acceptor charged surface area HACA-1/TMSA (Zefirov)*, *Maximum net atomic charge (Zefirov) for H atoms*, *LUMO + 1 energy (AM1)* (see also Table 1). The minimum

Table 1. Statistical summary and descriptor information for the general and improved ANN models of subchronic oral toxicity. D_{min}, D_{max}: minimum and maximum descriptor values for the training sets, *N*: number of data points, *R*: correlation coefficient between predicted and experimental property, *RMS*: root-mean squared error. All descriptor definitions and formulae are given in Supporting Information SI3.

ANN model	Input descriptors	D_{min}	D_{max}	*N* (tr, sel, test)	*R* (tr, sel, test)	*RMS* (tr, sel, test)
General (8-7-6-1)	LogP	−2.654	17.582	(90, 15, 15)	(0.781, 0.639, 0.731)	(0.129, 0.185, 0.119)
	Kier&Hall index (order 3)	0.056	15.771			
	Number of halogenide groups	0.000	6.000			
	Highest total interaction (AM1) for O–H bonds	−14.58	0.000			
	Average atom weight	4.746	24.797			
	HACA-1/TMSA (Zefirov)	0.000	0.067			
	Max net atomic charge (Zefirov) for H atoms	0.000	0.109			
	LUMO + 1 energy (AM1)	−2.470	3.628			
Improved (8-6-5-1)	LogP	−2.654	17.582	(73, 15, 15)	(0.886, 0.502, 0.823)	(0.111, 0.125, 0.114)
	Kier&Hall index (order 3)	0.000	15.771			
	Number of halogenide groups	0.000	6.000			
	Highest total interaction (AM1) for O–H bonds	−14.55	0.000			
	Average atom weight	4.746	24.797			
	HACA-1/TMSA (Zefirov)	0.000	0.067			
	Max net atomic charge (Zefirov) for H atoms	0.000	0.102			
	LUMO + 1 energy (AM1)	−0.866	3.642			

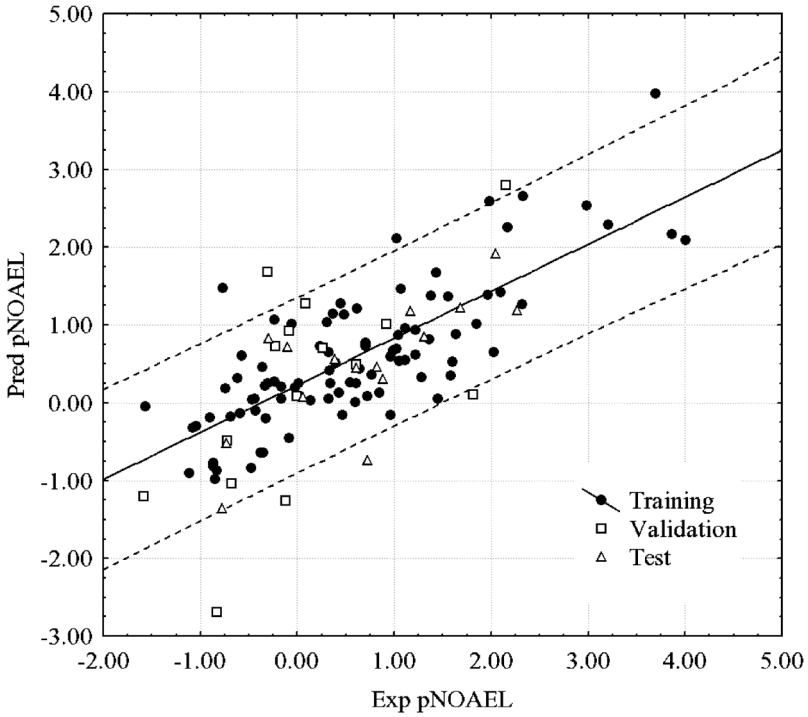

Figure 1. Experimental vs. predicted pNOAEL values based on the general ANN model for oral toxicity.

and maximum descriptor values for the whole training set are given in Table 1. These values were used to define the applicability domain of the ANN model as described in Section 2, Methods.

In this attempt for modeling, we also used LogP as a descriptor calculated by JLogP 2 program.[33] This descriptor, related to the lipophilicity of compounds was introduced after a chemical inspection has been performed on the remaining descriptors which were significant to the property. However, the majority of the descriptors tend to be related to reactivity (compounds stability), charge distributions and bulk characteristics of the compounds. It is known that the presence of halogens can make compounds more reactive. For instance, according to the ANN model developed, the value of the descriptor *Number of halogenide groups* would lead to increase of pNOAEL and thus lowers the NOEAL value. The next descriptor, *LUMO + 1* is the lowest unoccupied molecular orbital energy for orbital above the last unoccupied one. This parameter is related to the affinity potentials and thus again to the reactivity of the molecule and its stability. Hence, the descriptor increases the toxic effect in generally. The opposite trend can be observed in the case of *Highest total interaction (AM1) for O–H bonds* where it defines the stability of the O–H bonds. It is likely that the O-H bond is addressed to the most reactive centers surrounded by the media (water) where hydrogen acceptor/donor interactions take place. The importance of the hydrogen acceptor/donor interactions of the compounds is also featured by the descriptors *Hydrogen acceptor charged surface area HACA-1/TMSA (Zefirov)* and the *Maximum net atomic charge (Zefirov) for H atoms* where both parameters decreases the NOEAL value. Therefore, compounds with lower hydrogen acceptor/donor (charged hydrogens) ability would not produce adverse effect. The secondary effect related to the descriptors *Kier&Hall index (order 3)* and *Average atom weight* in the ANNs reflects the bulk properties for the chemicals. The *Kier&Hall index (order 3)* descriptor is a topological parameter which indicates the complexity and connectivity of the compounds. The more branched and heavier compounds generally will lower the NOEAL i.e. would increase the adverse effect.

In spite of the large diversity of the chemicals in the training set, the current ANN model can be applied for the prediction of NOAEL of new compounds that fall within the applicability domain.

An attempt was made to improve the general ANN model by analyzing the large outliers. From Figure 1, it can be seen that few compounds deviate significantly from the linear trend as well as few are outside the prediction bands. A leverage investigation of the deviations indicated that most of these outliers were addressed to sulfur-containing compounds. We then excluded the S-containing compounds and rebuilt the ANN model with the same descriptors. The reasons for the poor performance of the ANN model for these compounds are either poor parameterization in the AM1 method (S can promote different valence) or the experimental property for the extreme values reaches significant errors. By this exclusion procedure, the statis-

Table 2. The best ANN model for the subchronic inhalation toxicity (log(1/NOAEL)). D_{min}, D_{max}: minimum and maximum descriptor values for the training sets, *N*: number of data points, *R*: correlation coefficient between predicted and experimental property, *RMS*: root-mean squared error. All descriptor definitions and formulae are given in Supporting Information SI3.

ANN model	Input descriptors	D_{min}	D_{max}	*N* (tr, sel, test)	*R* (tr, sel, test)	*RMS* (tr, sel, test)
General (5-5-3-1)	Tot molecular 1-center E–E repulsion (AM1)/# of atoms	21.750	240.74	(51,10,10)	(0.877, 0.690, 0.882)	(0.133, 0.158, 0.119)
	Michalic MTI′	2.000	2246.0			
	Average nucleophilic reactivity index (AM1)	0.002	0.021			
	Gravitation index (all bonds) (AM1)	122.20	1458.5			
	HA dependent HDCA-2/SQRT(TMSA) (AM1)	0.000	0.197			

tical diversity of the set was reduced and led to an improved ANN model. Table 2 summarizes the statistical characteristics of the improved model together with its applicability domain parameters (D_{min}, D_{max}).

Altogether 10 different neural nets were developed in order to find the best architecture. The final best model (8-6-5-1) was first trained in 100 epochs by standard backprogation delta rule method and then in 98 epochs with Levenberg–Marquardt approach. As seen from the Table 1, the improved model has better statistical parameters and lower number of neurons than the general model. The predicted and experimental pNOAEL values for the improved model are graphically presented in Figure 2. The numerical values for the predicted and experimental data are collected in SI1. It can be noted from Figure 2 that few compounds are outside the prediction bands. The biggest outlier (circled, top right corner of the plot) is the compound dicofol (SI1, test set, No 3). This compound has high content of Cl atoms and possesses extreme experimental pNOAEL value. Additionally there are three outliers (circled, bottom, No 5, 9, 15 in SI1, selection set) which also possess high Cl content. The possible reasons for these compounds being large outliers is i) that the ANN model is not able to fit well these Cl compounds because of lack of explicit Cl descriptors (we sought generality of the model rather than specificity) or ii) the experimental error of the property reaches extreme magnitude especially for the extreme NOAEL values.

It should be noted that in these calculations we sought ANN models with as high *R* (*RMS* low) as possible for the

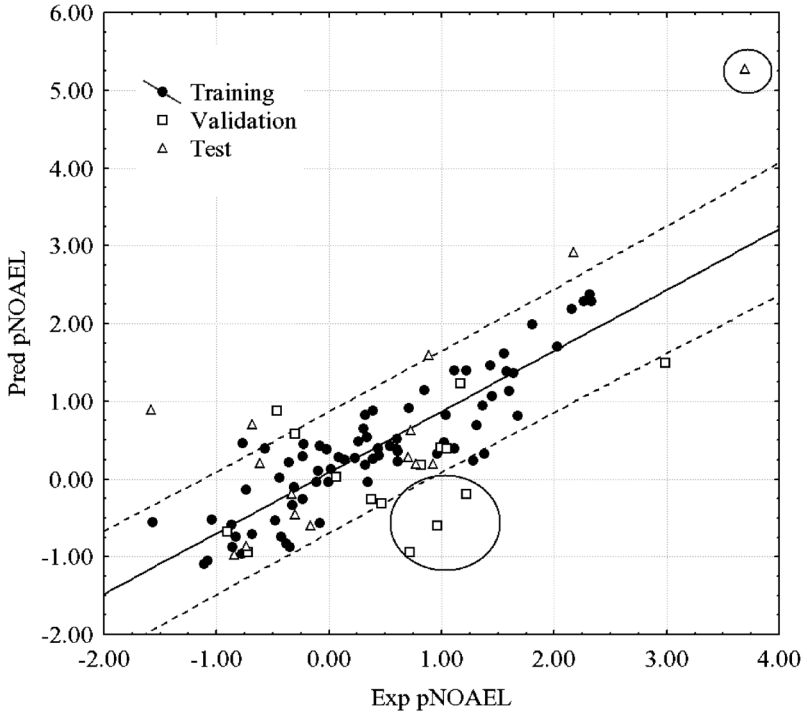

Figure 2. Experimental vs. predicted pNOAEL values based on the reduced ANN model for oral toxicity.

external test set to increase the predictive stability. In regulatory studies, both models can be used for the initial prediction and indication of NOAEL values. In the case of S-containing compounds the general ANN should be used for NOAEL predictions, otherwise the improved model is recommended.

3.2 ANN Model for Subchronic Inhalation Toxicity

In the modeling of subchronic inhalation toxicity (pNOAEL), we developed 14 different nets with various architectures tested on 51, 10, 10 data points for training, selection (validation) and test sets, respectively. The best input descriptors selected (as described in Sec. 2, Methods) were limited to five. The final best ANN model obtained is given in Table 2. The linear plot between the predicted and observed pNOAEL values based on this model is presented in Figure 3 and the respective numerical values are collected in supplementary information SI2. The model was first trained by 100 epochs using standard backprogation delta rule method and then 98 epochs with Levenberg–Marquardt approach for the final weight optimizations. For the sake of generality we limited the selection and test sets to 10 data points in each set since the total set is smaller compared to the oral toxicity set. The stopping criterion for the training was the *RMS* error of the selection set.

The ANN model presented in Table 2 possesses good statistical parameters, especially for the training and test sets. The respective graph in Figure 2 indicates that one compound deviates largely from the linear trend and the prediction bands. This compound is tetrahydrofuran (SI2, No 11, training set). It is a small oxygen containing compound. The reason of being large outlier might be that it does not fit well in the applicability domain of the remaining compounds in the training set or it has extreme NOAEL value (since it is highly volatile) and thus it could posses larger experimental error.

The most significant descriptors in the ANN model are *Gravitational index (AM1)* and *HA dependent HDCA-2/SQRT-(TMSA) (AM1)*. Both descriptors have positive correlation with the property that suggests its increase with the increased values of these descriptors. The *Gravitational index (AM1)* can be related to bulk characteristics of the compound governing specific interactions whilst the *HDCA-2/SQRT(TMSA) (AM1)* is related to hydrogen donor/acceptor ability of the molecules. The descriptor *Michalic MTI'* can

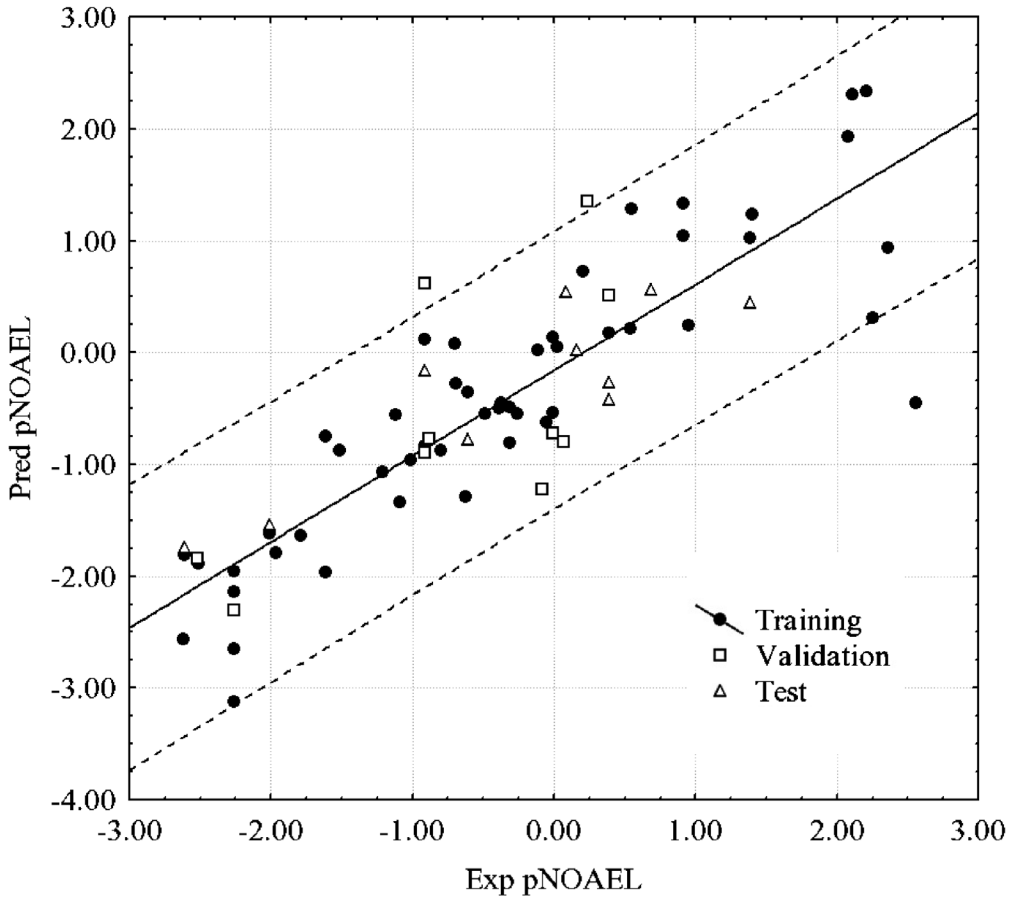

Figure 3. Predicted vs. observed pNOAEL values obtained by the ANN model for the inhalation toxicity.

also be addressed toward the bulk characteristics of the compounds. The last two descriptors, i.e. the *Average nucleophilic reactivity index (AM1)* and *Total molecular 1-center E–E repulsion (AM1)/# of atoms* can be related to the stability and reactivity of the compounds. It is very likely that once compound is in the respiratory tract (or even further in the blood stream), it is able to react with the surrounding media. The descriptor *Average nucleophilic reactivity index (AM1)* has negative correlation with the property thus its increase would decrease the pNOAEL. Moreover, the stability of the compounds encoded by transformation of the compounds encoded by the *Total molecular 1-center E-E repulsion (AM1)/# of atoms* can play important role in some metabolic pathways. This descriptor has positive correlation with the property.

4 Conclusions

In this study, we have successfully developed three ANN models for the prediction of subchronic oral and inhalation toxicities of NOAEL. Such challenging attempt is one of the first in this area where the above "difficult" properties are satisfactorily modeled. Despite the complicated nature of the phenomenon and the noncongeneric data set used, the ANN models can be used for an alternative method (nonanimal test) for initial prediction/assessment of NOAEL. For more accurate use of the models, the future predictions should also take into account the applicability domain of the models, for which we have defined quantitative limits based on the model descriptors.

It is worth mentioning that we have also tried multilinear regression models for these data sets. However, we did not succeed in this exercise since the statistical variability was too large for this approach (e.g. experimental pNOAEL for inhalation toxicity of the training set was in the interval [−2.61, 2.51]). In addition, the chemical diversity of the compounds and the related descriptors was too large to obtain simple (multi)linear relationships. Hence, we would stress the importance of the careful choice of the approach (whether ANN, MLR, genetic algorithms etc.) in order to tackle such difficult properties.

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