SAR and QSAR in Environmental Research
Vol. 17, No. 3, June 2006, 265–284

Taylor & Francis
Taylor & Francis Group

Validation of counter propagation neural network models for predictive toxicology according to the OECD principles: a case study

M. VRAČKO*††‡, V. BANDELJ§, P. BARBIERI¶, E. BENFENATI‖, Q. CHAUDHRY⊥, M. CRONIN$, J. DEVILLERS¥, A. GALLEGOS†, G. GINI††, P. GRAMATICA‡‡, C. HELMA§§, P. MAZZATORTA¶¶, D. NEAGU⊥⊥, T. NETZEVA†, M. PAVAN†, G. PATLEWICZ†, M. RANDIĆ‡, I. TSAKOVSKA† and A. WORTH†

†European Chemical Bureau, Institute for Health and Consumer Protection, European Commission Joint Research Centre, 21020 Ispra, Italy
‡Kemijski inštitut/National Institute of Chemistry, Hajdrihova 19, 1000 Ljubljana, Slovenia
§Istituto Nazionale di Oceanografia e di Geofisica Sperimentale, B. go Grotta Gigante-Briščiki 42/C, Sgonico-Zgonik (TS), Italy
¶Department of Chemical Sciences, University of Trieste, Via L. Giorgieri 1, I-34127 Trieste, Italy
‖Istituto di Ricerche Farmacologiche Mario Negri, Via Eritrea 62, 20157 Milano, Italy
⊥Central Science Laboratory, Sand Hutton, York YO41 1LZ, UK
$School of Pharmacy and Chemistry, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK
¥CTIS, 3 Chemin de la Gravière, 69140 Rillieux La Pape, France
††Department of Electronic and Informatics, Politecnico di Milano, Piazza L. da Vinci 32, 20133 Milano, Italy
‡‡Department of Structural and Functional Biology, University of Insubria, Via J.H. Dunant 3, I-21100 Varese, Italy
§§In silico toxicology, Talstrasse 20, D-79102 Freiburg, Germany
¶¶Department of BioAnalytical Science, Nestlè Research Center, Vers-Chez-les-Blanc 44, 1000 Lausanne 26, Switzerland
⊥⊥Department of Computing, School of Informatics, University of Bradford, BD7 1DP, UK

(*Received 19 January 2006; in final form 25 April 2006*)

The OECD has proposed five principles for validation of QSAR models used for regulatory purposes. Here we present a case study investigating how these principles can be applied to models based on Kohonen and counter propagation neural networks. The study is based on a counter propagation network model that has been built using toxicity data in fish fathead

*Corresponding author. Email: marjan.vracko@ki.si

SAR and QSAR in Environmental Research
ISSN 1062-936X print/ISSN 1029-046X online © 2006 Taylor & Francis
DOI: 10.1080/10659360600787650

minnow for 541 compounds. The study demonstrates that most, if not all, of the OECD criteria may be met when modeling using this neural network approach.

Keywords: Validation of QSAR models; Counter propagation neural network; Duluth database

1. Introduction

In recent years, (Q)SAR based predictive models have emerged as a powerful alternative to testing of chemical toxicity in animals. (Q)SAR models are usually developed using linear statistical algorithms and based on relatively simple sets of data. However, linear methods have limited utility in finding multi-dimensional relational patterns in more complex sets of data. In such situations, non-linear algorithms and soft-computing approaches have often proved to be more useful. The use of these new approaches has also opened the possibility of developing (Q)SAR models for a varied range of compounds, and using a multitude of molecular descriptors. Amongst these powerful data-mining techniques are the artificial neural networks. These are new and evolving computer technologies designed to learn from data in a manner emulating the learning pattern in the brain. An artificial neural network is defined by its architecture and learning strategy. The architecture shows how the segments of computer memory (weights) are connected together into a complex network. The learning strategy describes the algorithm of ''learning'', which is passing repeatedly through the data and adjusting its weights to minimize the error. Basically, there are two main groups of artificial neural networks, which differ in architecture and learning strategy: (i) unsupervised and supervised self organizing maps and (ii) supervised back-propagation artificial neural networks. The term 'unsupervised or supervised' serves to indicate whether descriptors (input variables) alone, or descriptors plus biological activities (output variables), participate in the training. In the back-propagation neural network, a training algorithm is defined with algebraic operations on the input and output side of a neuron. The input operation is a scalar product between descriptors and weights of a neuron and the output operation has the form of an activation function. Neural networks of different architectures and learning strategies are widely used in QSAR modelling. Due to the vast variety of neural network techniques it is impossible to present all of them in all their details in this article, and therefore readers are advised to consult references [1–4] for further reading. In this article we performed a case study focusing on unsupervised self organizing maps (Kohonen networks) and supervised self organizing maps (counter propagation neural networks), with a view to apply the OECD principles for validation of (Q)SAR models [5].

The Kohonen neural network represents a basic type of artificial neural networks [6, 7]. From a mathematical point of view, it involves mapping from a multi-dimensional descriptor space into a two-dimensional array of neurons. The mapping is a non-linear algorithm set as a sequence of learning epochs. An individual epoch runs in two steps. The first step is the competitive learning and the second step is the self organization of the map. The mapping preserves the topology of the original space but does not preserve the metric. This first statement essentially means

that similar objects, i.e. the objects located closely in descriptor space, are located close to each other on the map. On the other hand, in the two-dimensional map, the information about distances between objects in the descriptor space (the metric) is lost. There are at least two reasons to perform this transformation. Firstly, a trained map is a visualization of objects, which are originally located in a multi-dimensional data space. Our mind can not analyze objects in multi-dimensional space, but can easily recognize the similarity relationships in two-dimensional map (for example clusters). Secondly, a trained map is a mathematical model. After mapping the objects from spacious multi-dimensional space are relocated into a limited two-dimensional network. This means that the information originally spread over the entire descriptor space is overlapped and squeezed into a more limited two-dimensional network. A generalization of the Kohonen network is the counter propagation neural network (CP-NN), for which the architecture and learning strategy have been described in many textbooks and articles [4, 8, 9]. In addition to descriptor values, this technique introduces response (output) values into the modeling.

In the recent decades Kohonen neural networks and counter propagation neural networks have became an important tool in QSAR/QSPR modeling in the fields related to risk assessment and drug design [10–28]. They have been applied as modeling techniques beside the widely used Multiple Linear Regression. Due to their non-linear character, they are particularly useful for modeling non congeneric data sets. By non congeneric sets we mean the situation where groups of compounds within that sets are active on account of different mechanisms. Note, it is premature to state that in general neural network models outperform linear ones. Some articles have reported better statistical parameters for neural network models than linear ones [11, 12], and vice versa [13]. The counter propagation neural network with its multi-dimensional output-layer architecture is a powerful tool for classification, i.e., ordering of compounds into pre-defined classes [14–19]. In this case, the toxicity of a compound is defined as an affiliation to a particular class rather than as a lethal concentration [17, 18]. Examples have been reported using toxicity/carcinogenicity classes or modes of action as classes [19]. The Kohonen network is often used to perform a general analysis of data sets. Visual inspection of objects ordered in 2-D network facilitates easily recognition of similarity relationships among the objects, for example clusters of similar compounds [20–24]. Kohonen networks can also be used to split a data set into training and test sets in such a way that the all the information from that data set is univocally divided into all sub-sets [25, 26]. Detailed analysis of neighborhoods in networks can reflect common modes of activity, or highlight potential outliers. A graphic representation of an individual descriptor layer shows the distribution of descriptor values over the data set. Comparisons between descriptor layers and property layers may also indicate the relative importance of an individual descriptor within the model [27].

Kohonen network and CP NN have the potential to be used in chemical risk management, including priority setting, risk assessment, classification and labeling. For models to be used for regulatory purposes, it is important that they fulfill the criteria laid down under the OECD as far as possible (as described in section 2). The aim of this article is to review the counter propagation method in terms of its applicability to the OECD principles. As a case study we have developed a CP NN model for fish toxicity.

2. OECD principles for validation of (Q)SAR models used for regulatory purposes

The initiative for (Q)SAR validation principles was set during the 'Workshop on regulatory acceptance of (Q)SARs for human health and environmental endpoints', which was held on March 4–6, 2002 in Setubal, Portugal [28]. Participants from industry, government bodies, and academic institutions discussed the potential use of (Q)SAR models in chemical management and decision-making processes. They addressed many of the important questions associated with (Q)SAR modeling such as: the definition of endpoints and descriptors, the mechanisms of activity, the domain of models, the testing and validation of models, the availability of training data, and transparency of models. They agreed that there was a need to include (Q)SAR models into chemical management processes to accelerate the decisions, to lower costs and to minimize the number of animals sacrificed for testing purposes. To support the use of (Q)SAR models they adopted criteria (known as the six Setubal principles) for acceptance of (Q)SAR models in regulatory purposes.

At the 37th Joint Meeting of Chemicals Committee and Working Party on Chemicals, Pesticides & Biotechnology, held on 17–19 November in Paris, the OECD Member Countries discussed the Setubal conclusions. They generally supported the conclusions [5] but slightly re-formulated them into five adopted five principles, the so-called OECD principles for validation. (Q)SAR models considered for regulatory purposes should be associated with the following information:

(1) a defined endpoint
(2) an unambiguous algorithm
(3) a defined domain of applicability
(4) appropriate measures of goodness-of-fit, robustness and predictivity
(5) a mechanistic interpretation, if possible.

In a framework of OECD some of the QSAR models were evaluated accordingly to the principles [29].

According to Principle 1, a (Q)SAR should be associated with a 'defined endpoint', where endpoint refers to any physicochemical, biological or environmental effect. The intent of this principle is to ensure transparency in the endpoint being predicted, since a given endpoint could be determined by different experimental protocols and under different experimental conditions.

According to Principle 2, a (Q)SAR should be expressed in the form of an unambiguous algorithm. The intent of this principle is to ensure the transparency of modeling algorithm.

According to Principle 3, a (Q)SAR should be associated with a 'definite domain of applicability'. QSAR models are inevitably associated with limitations in terms of the types of chemical structures, physicochemical properties and mechanisms of actions.

According to Principle 4, a (Q)SAR should be associated with a 'appropriate measure of goodness-of-fit, robustness and predictivity'. This principle expresses the need to provide two types of information: the internal performance of a model (as expressed as goodness-of-fit and robustness) and the predictivity of model using an appropriate test set.

According to Principle 5, a (Q)SAR should be associated with a 'mechanistic interpretation', wherever such an interpretation can be made. It is not always possible,

from a scientific viewpoint, to provide a mechanistic interpretation of a given (Q)SAR. The intent of this principle is to ensure that there is an assessment of the possibility of a mechanistic association between the descriptors used in a model and the endpoint being predicted, and that any association is documented.

3. Case study

3.1 *Modeling*

As case study, we present a CP NN model to predict aquatic toxicity. It was developed using a set of 551 compounds from 'Duluth' database compiled by Russom *et al.* [31]. The dataset consists of acute fish toxicity values for fathead minnow expressed as a lethal concentration determinated after 96-hour exposure. The endpoint fulfills the first principle, since, it is referred to OECD Guideline 203. In addition, the compounds can be classified into four hazard classes representing the LC_{50} for fish [30, 32].

	Class 1	Class 2	Class 3	Class 4
Concentration ($mg\,L^{-1}$)	>100	10 to ≤ 100	1 to ≤ 10	≤ 1

The aquatic toxicity was measured as the acute fish toxicity The Duluth database has been extensively studied and many QSARs have been reported. An overview of QSAR studies is given in reference [31]. Different questions have been addressed such as: selection of relevant descriptors, selection of different subsets considering the mode of toxic action or assignment to a particular chemical class, as well as comparisons of different modeling techniques. For example, Huuskonen [33] applied electrotopological indices to study the aquatic toxicity (fathead minnow) of 140 organic chemicals comparing linear regression models with the error-back propagation neural network model. Kaiser and Niculescu [34] studied a set of 865 chemicals using functional group descriptors and probabilistic neural networks as the modeling technique. Martin and Young [35] studied the aquatic toxicity of 397 organic compounds, comparing multiple linear regression models and neural network models. Kaiser [36] gave an overview of using neural networks in the modeling of different toxicology endpoints. Toropov and Toropova [37] investigated the toxicity of 69 benzene derivatives using optimized correlation weight indices and Morgan extended connectivity indices. Toropov and Benfenati [38] used Morgan extended connectivity and nearest neighboring codes as local graph invariants in a study of 51 aldehydes. Pintore *et al.* [39] applied a combination of several advanced techniques including self organizing maps, genetic algorithm and adaptive fuzzy partitioning to model fish toxicity for 568 compounds from the Duluth database. A further overview of other advanced modeling techniques is presented in reference [40]. Mazzatorta *et al.* [20] studied a set of 562 compounds with Kohonen and counter propagation neural networks. Barbieri *et al.* [25] investigated a set of 568 compounds using Kohonen and CP neural networks. Several questions were discussed such as analysis of outliers, neighborhoods in Kohonen network, division into training and test set, visualization of data. Additional readings on QSARs for aquatic (fish) toxicity and their use in chemical risk management can be found in references [41–47].

3.2 *Descriptors*

The descriptors for the model described below were calculated using the MDL QSAR software program [48]. The program selected 22 descriptors, which are shown in table 1.

4. Modeling process

4.1 *Data preprocessing*

The descriptors were normalized to the interval [0, 1] according to the following equation:

$$x_1^{\text{new}} = \frac{x_1 - x_1^{\min}}{x_1^{\max} - x_1^{\min}},$$

x_1^{new} – *transformed descriptor*, $x_1^{\min/\max}$ – minimal/maximal *value of descriptor*.

The descriptors together with their minimal and maximal values are given in table 1.

4.2 *CP NN architecture*

The CP NN developed consists of two layers, the input or Kohonen layer and the output layer [2–5]. This is a two dimensional network of neurons (25×25), which are

Table 1. Descriptors used for modeling (calculated and selected with MDL program). The minimal and maximal values were used for rescaling procedure.

	Descriptor's abbreviation	*Meaning of descriptor*	*Min.*	*Max.*
1	SsCH3_acnt	Count of all (–CH3) groups in molecule	0	9
2	SdCH2_acnt	Count of all (=CH2) groups in molecule	0	2
3	SssCH2_acnt	Count of all (–CH2–) groups in molecule	0	15
4	SddC_acnt	Count of all (=C=) groups in molecule	0	2
5	SaasC_acnt	Count of all (aasC) groups in molecule	0	10
6	SssssC_acnt	Count of all (>C<) groups in molecule	0	3
7	SdsN_acnt	Count of all (=N–) groups in molecule	0	2
8	SaaN_acnt	Count of all (aaN) groups in molecule	0	3
9	SdaaN_acnt	Count of all (daaN) groups in molecule	0	1
10	SsOH_acnt	Count of all (–OH) groups in molecule	0	3
11	SsF_acnt	Count of all (–F) groups in molecule	0	6
12	SssS_acnt	Count of all (–S–) groups in molecule	0	2
13	SdssS_acnt	Count of all (=S<) groups in molecule	0	1
14	SsBr_acnt	Count of all (–Br) groups in molecule	0	5
15	xch6	Simple 6th order chain chi index	0	0.3062
16	xch8	Simple 8th order chain chi index	0	0.0833
17	k0	Zero Order Kappa Shape Index, encodes the number of vertex symmetry classes in the graph, increases with an increase in the number of symmetry classes in the graph or decreases with increasing symmetry	0	46.4986
18	k1	First Order Kappa Shape Index, encodes the degree of cyclicity in the graph, decreases as graph cyclicity increases	2	27.5853
19	ka3	Third Order Kappa Alpha Shape Index	0	18.1633
20	logP	Calculated value of log P	−1.8943	7.3877
21	ovality	Ovality of molecule	1.0947	1.9773
22	SpcPolarizability	Specific polarizability of molecule (Polarizability/Volume)	0.0108	0.1408

vectors W_j $(w_{j1}, w_{j2}, \ldots, w_{jm})$. Here, W_j refers the neuron, $w_{j,i}$ are components of the vector (weights), and m is the dimension of the vector. The dimension of the input layer is equal to the dimension of the descriptor space (in our case, 22). The output layer is located beneath the Kohonen layer having the same arrangement of neurons. The dimension of the output layer is equal to the number of output (property) variables. In this study, the number of output variables is five, one corresponding to the concentration whilst the other four belong to the four classes of toxicity (see figure 1).

4.3 *Initialization of model*

The first step in the learning of a model is the initialization of weights, which are initialized as random numbers. The random number generator is described in Lewis *et al.* [49].

4.4 *Learning*

4.4.1 Selection of winning neuron. A vector of input variables (descriptors) is presented to all neurons. According to equation (1), the algorithm selects the neuron whose weights are closest to the input variables (winning neuron).

$$\delta_j = \sqrt{\sum_{i=1}^{m}(x_{si} - w_{ji})^2} \quad \delta_c = \min(\delta_1, \delta_2, \ldots \delta_j, \ldots \delta_n,) \Rightarrow W_c \tag{1}$$

4.4.2 Modification of weights. The weights of the winning neuron are modified to the values of the input variables ($\delta_c = 0$). Simultaneously, the weights of the neighboring neurons are modified to become similar to the input variables (equation (2)).

$$w_{ji}^{\text{new}} = w_{ji}^{\text{old}} + \eta(t) \times b(d_c - d_j) \times (x_{si} - w_{ji}^{\text{old}}) \tag{2}$$

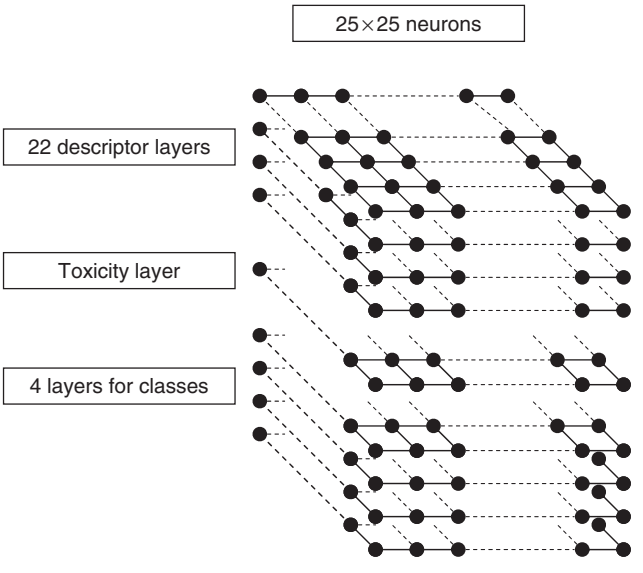


Figure 1. Architecture of counter propagation neural network.

Parameter η determines the rate of learning; it is maximal at the beginning ($t=1$, $\eta=a_{max}$) and minimal at the end of the learning procedure ($t=t_{max}$, $\eta=a_{min}$). The function $b(\cdot)$ in equation (2) describes how the correction of the weights w_{ji} decreases with the increasing topological distance between the central neuron and the neuron being corrected. Index j specifies individual neuron and runs from 1 to n. The learning runs over several epochs ($t=m$) until the weights are stabilized.

4.4.3 Learning in the output layer. This step is a supervised learning because target values are required for each input. The positions of objects are projected from the input to the output layer. The weights in the output layer are modified in such a way that the weights on projected positions correspond to the output values. The weights in the neighborhood are modified according to equation (3). In this way the response surface is constructed.

$$out_{ji}^{new} = out_{ji}^{old} + \eta(t) \times b(d_c - d_j) \times (T_{si} - out_{ji}^{old}) \quad (3)$$

We used in-house developed FORTRAN based computer programs for modeling [50]. The model was developed in accordance with the technical parameters described in table 2.

4.4.4 Classification. With the special construction of the output layer, the CP NN becomes a powerful tool in classification. For a property described with n classes, n layers must be added in the output layer. Affiliation of a compound to one of the four classes as defined in the previous section is described by the four dimensional vector. One element of the vector is equal to one, i.e., those to which the class a compound belongs and other elements are zero. With a multidimensional description of the classes a CP NN with four output layers can be constructed. During training all the output layers are trained independently. The prediction result is also a four dimensional vector with elements set as real numbers. Each number expresses the affiliation of a predicted compound to the corresponding class. In the predicted classes different situations can occur. Firstly, one element is larger than others. In this case, the predicted compound is unambiguously classified. Secondly, a compound is classified into two neighboring classes. This means that the model classifies the compound somewhere in between. Thirdly, a compound is classified into different classes with about the same affiliation.

Table 2. Technical parameters used for modeling. Parameters are associated with the program kctrf [50].

Parameter	*Value*	*Range*
Random routine used	Ref. [49]	
Randomization of object sequence order	NO	YES/NO
Number of neurons in x direction (DX)	25	Depend on program option
Number of neurons in y direction	25	Depend on program option
Number of weights in each neuron	27	No. of descriptors + targets
Toroid boundary condition	NO	YES/NO
Type of neighborhood correction	Triangular	
Furthest neuron for correction	30	1–DX
Maximal correction factor (MCF)	0.50	
Minimal correction factor	0.01	0–MCF
Number of epochs – m	100	No limits

It means that the model can not classify the compound. We know *a priori* that the model does not work for this compound and this in itself is a very valuable information.

5. Testing and validation

5.1 *Goodness of fit (recall ability test)*

In this test the prediction is made for the compounds in the training set. This shows how good the model recognizes the training objects. It is recognized that the recall ability test typically overestimates the prediction ability of a neural network models [1, 3, 10, 12]. Thus, the recall ability test results should be considered in combination with other tests. Table 3 shows correlation coefficients for models trained with different number of epochs. The correlation coefficient is stabilized after 200 training epochs, however, a further test shows that the model is already over-trained. After other tests the model trained with 100 epochs, was selected as the final one. Figure 2(a) shows the recall ability regression line. Classification results are shown in table 4. It can be seen that 79.49% of the compounds are classified correctly, and 94.55% of compounds are either classified correctly or misclassified for one class. The compounds over-classified, (the predicted class is higher than target class) are 10.07% whereas 9.44% of compounds are under-classified (the predicted class is lower than target class).

5.2 *Leave-one-out test, leave-20% out and predictions for test set*

In leave-out tests, the data set is divided into training sets and test sets. In consecutive steps of testing, the models are built with training sets and applied on test sets. The test provides information on the predictive power of the model, but also information on the consistency of the training data. We applied leave-one-out and leave-20%-out tests. The correlation coefficients are shown in table 3. After 150 epochs of training the correlation coefficient drops indicating that the models are over-trained. The regression line for model trained with 100 epochs is shown in figure 2(b). Similar trends can be observed in a 20% leave-out test. Table 4 shows the classification results. In the leave-one-out-test, 55.90% of compounds are classified correctly, 23.77% and 20.33% are over-classified and under-classified, respectively. In the leave-20%-out test, 47.91% of compounds are classified correctly, whereas 12.18% and 39.91% are over-classified and under-classified, respectively.

Table 3. Correlation coefficients for different tests for models trained with 50–300 epochs.

No. of learning epochs	*Recall ability*	*Leave-one-out*	*Leave-20%-out*	*Test set*
50	0.861	0.730	0.699	0.918
100	0.898	0.748	0.708	0.923
150	0.900	0.748	0.723	0.895
200	0.930	0.730	0.707	0.892
300	0.930	0.728	0.691	0.868

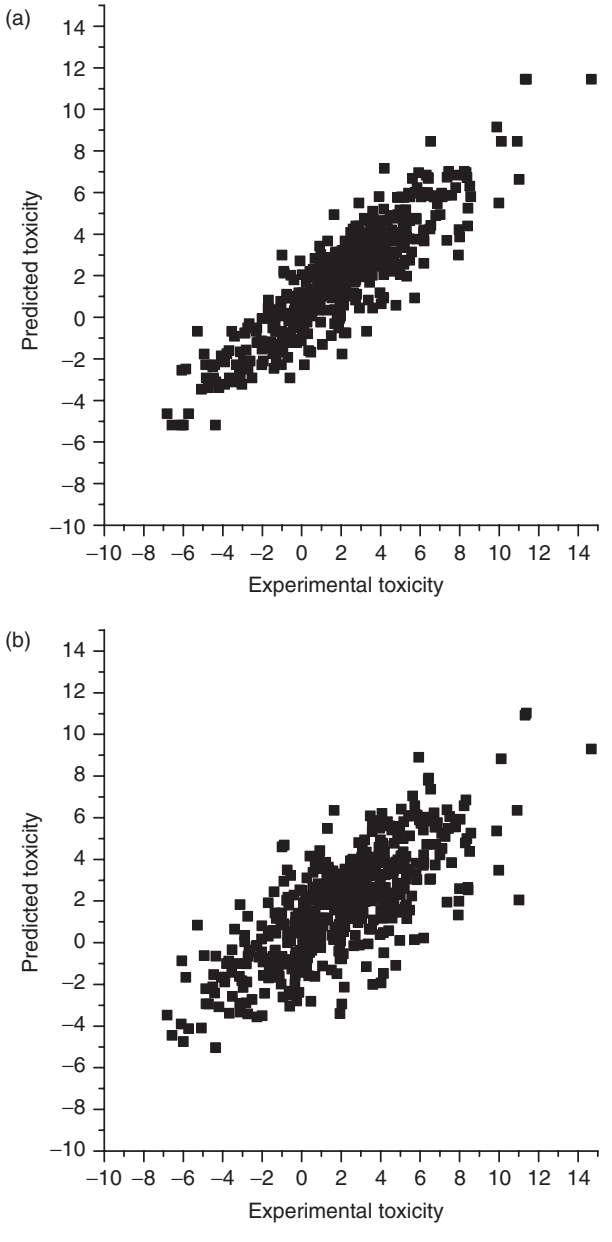


Figure 2. Regression lines for recall ability test (a) and for leave-one-out test (b).

5.3 *Predictions for the external test set*

Before the modeling work, 10 compounds were selected as an external test set. These 10 compounds were selected randomly from different regions of the descriptor space, and were never used in the training sets.

Table 4. Classification results for different tests. Compounds were classified into four classes according to fish toxicity.

	Recall ability test		*Leave-one-out test*		*Leave-20%-out test*	
Target class–predicted class	*# of comp.*	*%*	*# of comp.*	*%*	*# of comp.*	*%*
−3	1	0.18	2	0.36	1	0.18
−2	16	3.27	28	5.44	4	1.11
−1	40	7.62	97	17.97	58	10.89
0	436	79.49	306	55.90	262	47.91
1	39	7.44	95	17.60	105	19.42
2	8	1.81	11	2.36	70	13.07
3	1	0.19	2	0.37	41	7.42

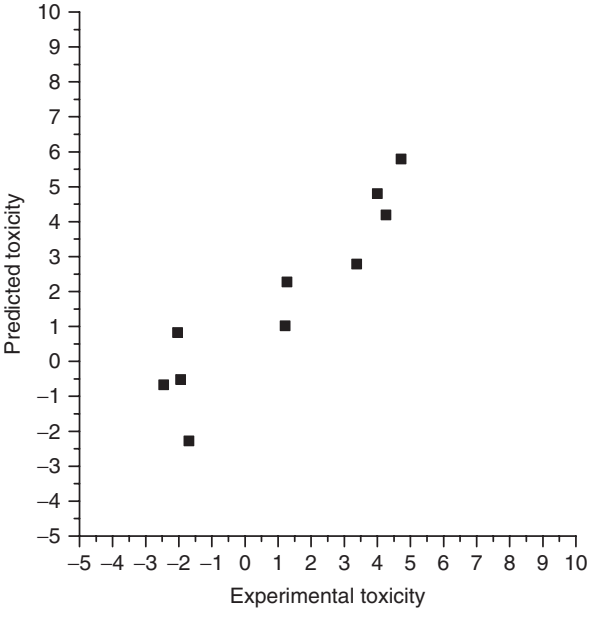


Figure 3. Regression line for the test set of ten compounds.

5.3.1 Prediction of toxicity values and classes. The regression line showing predicted versus experimental values is shown in figure 3. The statistical parameters of the line are: $r = 0.923$, standard deviation SD = 1.187, the intercept on y axis $a = 0.803$, the slope $b = 1.027$. Individual predictions and classification results are shown in table 5. Six of compounds (2-cyanopyridine, diethylamine, 2-octanone, 3,5-diiodo-4-hydroxy-benzonitrile, 2,2-dimethyl-1-propylamine, [*1*(*r*)-*endo*]-(+)-*3*-Bromocamphor) are classified in the correct class with an affiliation larger than 0.6. Dibutyl adipate and 2′,3′,4′-trichloroacetophenone are classified in two neighboring classes. On the other hand, the isopropyl ether is classified into the first and third class with affiliation numbers 0.5 and 0.4, respectively and the *p-tert*-butylphenol is classified into classes 1 to 3. For the last two cases *we know* that the model is poorly appropriate in predicting

Table 5. Experimental and predicted toxicity values and classes for ten compounds of the test set.

Compound	*Exp. Tox.*	*Pred. tox.*	*Exp. class*	*Pred. class*
Isopropyl ether	−2.0402	0.8248	1.0	0.5
				0.1
				0.4
2-Cyanopyridine	−1.9421	−0.5170	1.0	0.8
				0.2
Diethylamine	−2.4587	−0.6684	1.0	0.7
				0.1
				0.3
Dibutyl adipate	4.2624	4.1969		
				0.4
			1.0	0.5
				0.1
2-Octanone	1.2702	2.2745		
			1.0	0.6
				0.4
p-tert-Butylphenol	3.3731	2.7875		0.2
				0.3
			1.0	0.4
				0.1
3,5-Diiodo-4-hydroxybenzonitrile	3.9990	4.8021		
				0.1
			1.0	0.6
				0.3
2,2-Dimethyl-1-propylamine	−1.6954	−2.2796	1.0	0.8
				0.2
2′,3′,4′-Trichloroacetophenone	4.7162	5.7960		
				0.1
			1.0	0.1
				0.8
[1(r)-endo]-(+)-3-Bromocamphor	1.2162	1.0152		0.3
			1.0	0.7

these toxicities. It is necessary to emphasize that the predictions of toxicity values and classes are independent, because the individual input layers are constructed independently. In a very peculiar situation, the predicted value is correct, but the compound is ambiguously classified.

5.3.2 Neighbors and clusters. The prediction of values for a new compound in the CP NN model runs in two phases. First, the algorithm allocates the winning neuron, which is found as described above. Second, its position is projected to the output layer where the predicted values are found. The winning neuron and the neighboring neurons provide additional information to the prediction. The compounds of training set, which are located on those neurons, determine the prediction. When they are similar to a new compound, they may indicate a similarity in the mechanism of activity. For the case study presented, table 6 shows the compounds in the test set, the positions of the winning neurons and the compounds in the training set located on the neighboring neurons.

Table 6. Distances to winning neuron, positions in network and closest neighbors in the network for ten compounds of the test set. The distance to the winning neuron is a measure how good a prediction fits to the general framework of model. The position and neighbors determinate the prediction. The corresponding toxicity values are in parentheses.

Compound	*Dist. to WN*	*Position in NN*	*Neighbors*
Isopropyl ether	0.1390	25,21	2,5-Dimethyl-2,4-hexadiene (3.373) 4-Methyl-2-pentanone (−1.685) 5-Methyl-2-hexanone (−0.331) 4-Dimethylamino-3-methyl-2-butanon (2.721) Fensulfothion (1.965) 3-Methyl-2-butanone (−2.306) Tetraethyltin (−1.181)
2-Cyanopyridine	0.1314	1,20	2-Picoline (−2.265) 3-Picoline (−0.436) 4-Picoline (−1.465) 3-Pyridinecarboxaldehyde (1.877) Pyridine (−0.293)
Diethylamine	0.0904	24,19	Diethyl ether (−3.542) *(+−)-sec*-Butylamine (−1.324) Isovaleraldehyde (3.277) 2,4-Pentanedione (−0.558) Ethyl acetate (−0.959) 3-Pentanone (−2.884) 2-Methylbutyraldehyde (2.156) 2-Pentanone (−2.667) 2-Methylvaleraldehyde (1.673) Methyl acetate (−1.463) 2-Butanone(−3.799)
Dibutyl adipate	0.0890	21,1	Diethyl sebacate (4.561) *tris*(2-Butoxyethyl)Phosphate (3.572) Tributyl phosphate (3.187)
2-Octanone	0.0754	19,7	Butyl ether (1.394) 1-Nonanol (3.231) Tripropylamine (1.035) 2-Nonanone (2.236) 1-Bromoheptane (4.803)
p-tert-Butylphenol	0.2166	5,3	Flavone (4.150) *p-(tert*-butyl*)*-phenyl-*n*-Methylcarbamate (3.031) Carbofuran (5.569) *p-(tert*-butyl*)*Benzamide (1.715)
3,5-Diiodo-4-hydroxy-benzonitrile	0.0789	14,19	2,3,4,6-Tetrachlorophenol (5.417) Pentachlorophenol (7.090) 2,3,4,5-Tetrachlorophenol (6.338) 2,4,6-Triiodophenol (5.966) 2,4,6-Trichlorophenol (3.071)
2,2-Dimethyl-1-propylamine	0.1662	25,23	5,5-Dimethyl-1,3-cyclohexanedione (−4.408) 5,5-Dimethylhydantoin (−4.856) 3,3-Dimethyl-2-Butanone (0.141) *tert*-butyl Methylether (−2.031) *tert*-butyl Acetate (−1.035)
2′,3′,4′-Trichloroacetophenone	0.1230	11,18	1,3,5-Trichloro-2,4-dinitrobenzene (7.109) 1,3-Dichloro-4,6-dinitrobenzene #1 (8.425)

(*Continued*)

Table 6. Continued.

Compound	*Dist. to WN*	*Position in NN*	*neighbors*
[1(r)-endo]-(+)-3-Bromocamphor	0.3203	25,25	*tert*-butyl Sulfide (1.615) *tert*-Octylamine (1.659) 1,8-Diamino-*p*-menthane (0.959) 2,2,5,5-Tetramethyltetrahydrofuran (−0.270) *(1s)-(−)*-Camphor (2.192) Cineole (0.414) 1,1,1-Trichloro-2-methyl-2-Propanol(hydrate) (0.273) *[(1s)-endo]*-(−)-Borneol (0.961)

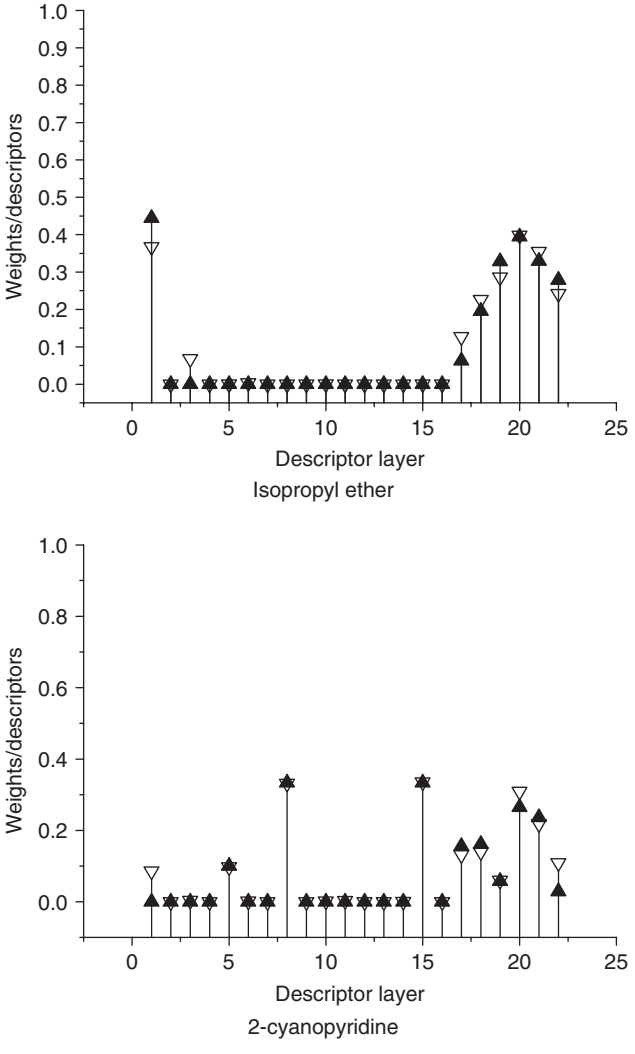


Figure 4. Values of descriptors (▲) and weights (▽) on the winning neurons for ten compounds of the test set. One obtains detailed information how good the predicted compound fits to the model.

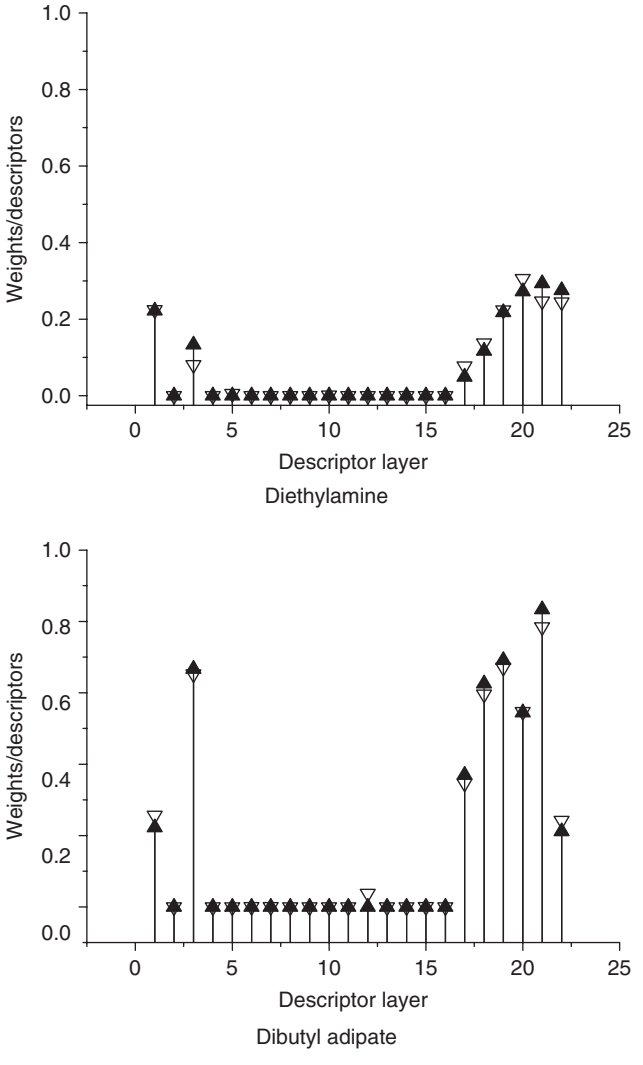
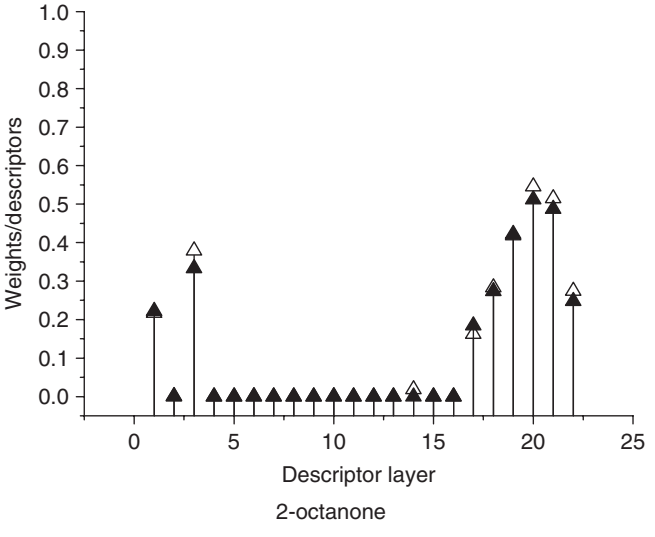


Figure 4. Continued.

5.3.3 The distance between the winning neuron and descriptors. In Kohonen network and CP NNs the values of weights generally determine the domain of the model. This is to emphasize that the values of the weights are not equal to the values of the descriptors, as the weights were modified during the training and they adopted values 'similar to the descriptors'. If a new compound is presented to the model for prediction, and it is situated on the winning neuron, the distance between the descriptors and the winning neuron can be considered as a measure of how good a new compound fits into the domain of the model. This information will fulfill the requirement under OECD principle 3 (domain of applicability). In table 6 we report the distances to the winning neurons for all 10 compounds of the test set. It is an



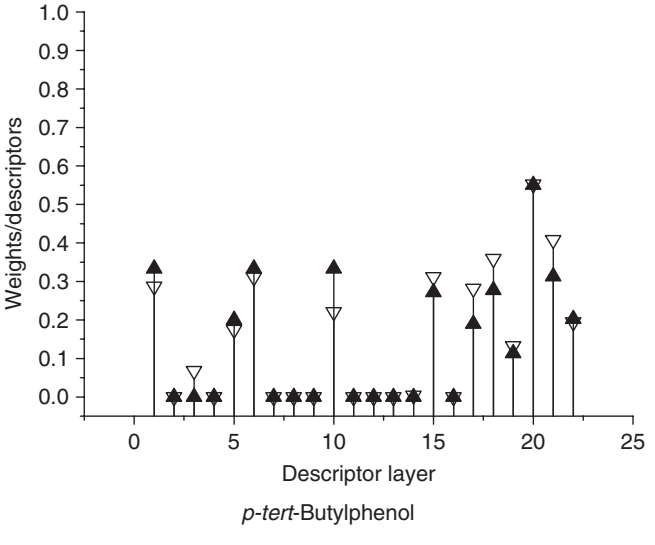


Figure 4. Continued.

interesting observation that *p-tert*-butylphenol, which was not properly classified, shows a longer distance to the winning neuron. This emphasizes how it is not entirely in the domain of the model. Figure 4 shows further details from the model in terms the individual values of weights and descriptors for the compounds in the external test set. This is to emphasize how the distance between the winning neuron and descriptors represents additional information in the prediction. The prediction can be good even if the distance is large, or vice versa. Ultimately it is up to the user on how to interpret this additional information.

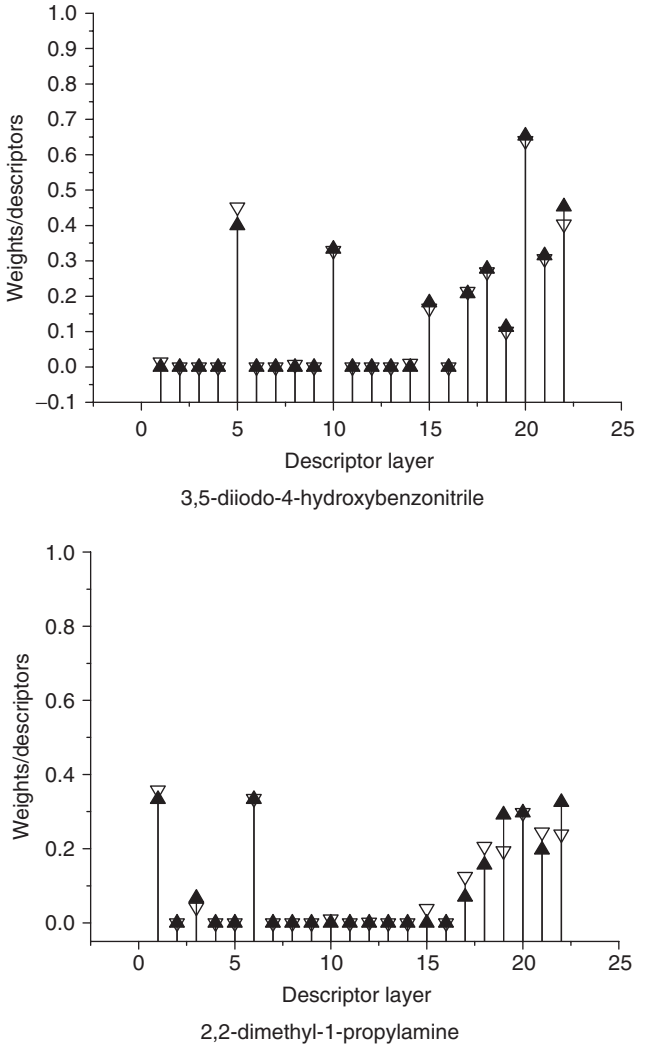


Figure 4. Continued.

6. Conclusions

In this article we have presented a counter propagation neural network model for fish toxicity that has been built using a set of 541 compounds from the Duluth database. The case study presented here, clearly demonstrates that a (Q)SAR model can be derived and validated using a CP NN approach whilst still satisfying most, if not all of the OECD principles for validation of (Q)SAR models. These findings are likely to open up further opportunities for using this powerful technique for developing and validating (Q)SAR models for compounds from different chemical classes, and using large sets of descriptor data. However, KSOM and CP NN represent a class of ANN with relatively simple architecture and learning algorithm. It is noteworthy that models

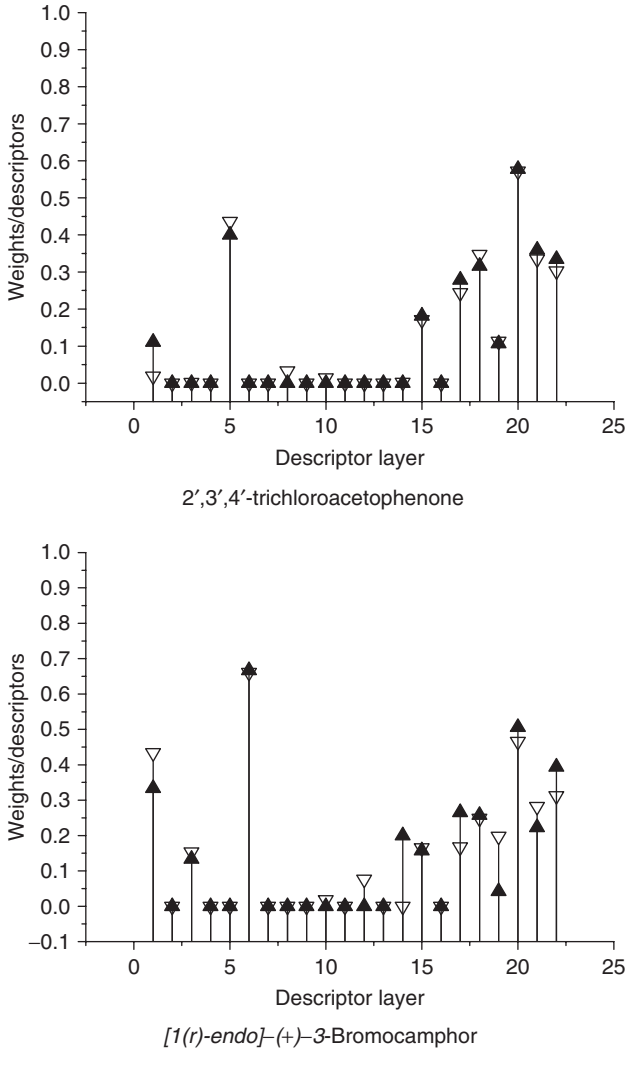


Figure 4. Continued.

built with others ANN techniques remain to be validated according to the OECD principles.

Acknowledgement

MV acknowledges receipt of Senior Researcher Visitor grant from the ECB, Institute for Health and Consumer Protection, European Commission Joint Research Centre. C. Helma was funded by a grant (1328-179) of the Centre for Documentation and Evaluation of Alternatives to Animal Experiments (ZEBET) of the Federal Institute for Risk Assessment (BfR).

References

[1] J. Devillers. *Neural Networks in QSAR and Drug Design*, Academic Press, London (1996).
[2] J. Zupan, J. Gasteiger. *Neural Networks in Chemistry and Drug Design*, Wiley-VCH, Weinheim (1999).
[3] M. Novič, M. Vračko. In *Nature-Inspired Methods in Chemometrics: Genetic Algorithms and Artificial Neural Networks*, R. Leardi (Ed.), pp. 231–256, Elsevier, Amsterdam (2003).
[4] J. Dayhof. *Neural Network Architecture, An Introduction*, p. 192, Van Nostrand Reinhold, New York (1990).
[5] The principles for establishing the status of development and validation of (quantitative) structure-activity relationships [(Q)SARs]. OECD document ENV/JM/TG(2004)27.
[6] T. Kohonen. *Self-Organizing Maps*, Springer, Berlin (2000).
[7] Kohonen's group of Helsinki University of Technology. Available online at: www.cis.hut.fi/research/som-research/nnrc-programs.shtml
[8] R. Hecht-Nielsen. *Appl. Opt*., **26**, 4979 (1987).
[9] K.L. Peterson. *J. Chem. Inf. Comput. Sci*., **35**, 896 (1995).
[10] M. Vračko. *Curr. Comp.-Aid. Drug Des*., **1**, 73 (2005).
[11] M. Novičž, Ž. Nikolovska-Coleska, T. Šolmajer. *J. Chem. Inf. Comput. Sci*., **37**, 990 (1997).
[12] B. Bienfait. *J. Chem. Inf. Comput. Sci*., **34**, 890 (1994).
[13] M. Vračko, D. Mills, S.C. Basak. *Environ. Toxicol. Pharmacol*., **16**, 25 (2004).
[14] H. Satoh, O. Sacher, T. Nakata, L. Chen, J. Gasteiger, K. Funatsu. *J. Chem. Inf. Comput. Sci*., **38**, 210 (1998).
[15] L. Chen, J. Gasteiger. *J. Am. Chem. Soc*., **119**, 4033 (1997).
[16] P. Gramatica, N. Navas, R. Todeschini. *Trends. Anal. Chem*., **18**, 461 (1999).
[17] M. Vračko, M. Novič, J. Zupan. *Anal. Chim. Acta*, **384**, 319 (1999).
[18] M. Vračko. *SAR & QSAR Environ. Res*., **11**, 103 (2000).
[19] S. Spycher, E. Pellegrini, J. Gasteiger. *J. Chem. Inf. Model*., **45**, 200 (2005).
[20] P. Mazzatorta, M. Vračko, A. Jezierska, E. Benfenati. *J. Chem. Inf. Comput. Sci*., **43**, 485 (2003).
[21] M. Novič, M. Vračko. *Chemom. Intell. Lab. Systems*, **59**, 33 (2001).
[22] J. Kawakami, K. Hoshi, A. Ishiyama, S. Miyagishima, K. Sato. *Chem. Pharm. Bull*., **52**, 751 (2004).
[23] I. Valkova, M. Vračko, S.C. Basak. *Anal. Chim. Acta*, **509**, 179 (2004).
[24] P. Gramatica, V. Consonni, M. Pavan. Structural-toxicity mode of action similarity analysis by Kohonen artificial neural network (K-ANN), presented at 14th Annual Meeting SETAC-Europe, Prague, CR, 18–23 April (2004).
[25] P. Barbieri, N. Piclin, A. Szymoszek, M. Novič, M., Vračko, E. Benfenati. QSTR modelling of acute toxicities on Fathead Minnow (*Pimephales promelas*) by counter propagation neural networks, presented at Meeting of Slovenian Chemical Society 2001. Maribor, SLO, 20–21 September (2001).
[26] P. Gramatica, P. Pilutti, E. Papa. *J. Chem. Inf. Comput. Sci*., **44**, 1794 (2004).
[27] A. Jezierska, M. Vračko, S.C. Basak. *Molec. Diver*., **8**, 371 (2004).
[28] Regulatory acceptance of QSARs for human health and environmental endpoints. Proceeding of Workshop, Setubal, Portugal, March 2002.
[29] Test guidelines programme. OECD document ENV/JM/TG(2004)27/ANN.
[30] Guidance Document on the Use of Harmonized System for the Classification of Chemicals which are Hazardous for the Aquatic Environment (2001).
[31] C.L. Russom, S.P. Bradbury, S.J. Broderius, D.E. Hammermeister, R.A. Drummond. *Environ. Toxicol. Chem*., **16**, 948 (1997).
[32] Available online at: www.laus.de/en/leistungen/oekotoxicologie/wassergefaerdungsklasse/
[33] J. Huuskonen. *Chemosphere*, **50**, 949 (2003).
[34] K.L.E. Kaiser, S.P. Niculescu. *Chemosphere*, **38**, 3237 (1999).
[35] T.M. Martin, D.M. Young. *Chem. Res. Toxicol*., **14**, 1378 (2001).
[36] K.L.E. Kaiser. *J. Mol. Struct. (Theochem)*, **622**, 85 (2003).
[37] A.A. Toropov, A.P. Toropova. *J. Mol. Struct. (Theochem)*, **578**, 129 (2002).
[38] A.A. Toropov, E. Benfenati. *J. Mol. Struct. (Theochem)*, **676**, 165 (2004).
[39] M. Pintore, N. Piclin, E. Benfenati, G. Gini, J.R. Chretien. *QSAR Comb. Sci*., **22**, 210 (2003).
[40] E. Benfenati. Modelling aquatic toxicity with advanced computational techniques: Procedure to standardize data and compare models. In *Knowledge Exploration in Life Science Informatics: International Symposium KELSI 2004*, J.A. Lopez, E. Benfenati, W. Dubitzky (Eds), pp. 235–248, Springer-Verlag GmbH, Berlin (2004).
[41] M.T.D. Cronin, J.C. Dearden. *Quant. Struct.-Act. Relat*., **14**, 1 (1995).
[42] I. Lessigiarska, A.P. Worth, T.I. Netzeva. Comparative review of QSARs for acute toxicity. EUR 21559 EN, © European Communities (2005).
[43] G.M. Rand, P.G. Wells, L.S. McCarty. Introduction to aquatic toxicity. In *Fundamentals of Aquatic Toxicology: Effects, environmental Fate and Risk Assessment*, G.M. Rand (Ed.), pp. 3–66, Taylor & Francis, Washington, DC (1995).

[44] J. Devillers, D. Domine. *SAR QSAR Environ. Res.*, **10**, 61 (1999).
[45] J. Devillers, J. Flatin. *SAR QSAR Environ. Res.*, **11**, 25 (2000).
[46] J. Devillers. *SAR QSAR Environ. Res.*, **11**, 397 (2001).
[47] P. Gramatica, F. Villa, E. Papa. *J. Chem. Inf. Model.*, **45**, 1256 (2005).
[48] MDL QSAR Version 2.2.0.0.446 (SP1) Copyright © 2002–2004.
[49] P.A.W. Lewis, A.S. Goodman, J.M. Miller. *IBM Sys. J.*, **8**, 136 (1969).
[50] Program for counter propagation modeling, kctrf.f ©, National institute of chemistry, Ljubljana, Slovenia.