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A regression-based QSAR-model to predict acute toxicity of aromatic chemicals in tadpoles of the Japanese brown frog (*Rana japonica*): calibration, validation, and future developments to support risk assessment of chemicals in amphibians

Andrey A. Toropov[a,§], **Matteo R. Di Nicola**[b,c,§,*], **Alla P. Toropova**[a], **Alessandra Roncaglioni**[a], **Edoardo Carnesecchi**[d,e], **Nynke I. Kramer**[c,d], **A.J. Antony J. Williams**[f], **Manuel E. Ortiz-Santaliestra**[g], **Emilio Benfenati**[a], **Jean-Lou C. M. Dorne**[h]

[a]Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Via Mario Negri 2, 20156 Milano, Italy

[b]Unit of Dermatology and Cosmetology, IRCCS San Raffaele Hospital, Via Olgettina 60, 20132 Milan, Italy

[c]Toxicology Division, Wageningen University, PO Box 8000, 6700 EA Wageningen, The Netherlands

[d]Institute of Risk Assessment, Utrecht University, PO Box 80177, 3508 TD Utrecht, The Netherlands

[e]Evidence Management Unit, European Food Safety Authority (EFSA), Via Carlo Magno 1A, 43126 Parma, Italy

[f]Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency (U.S. EPA), Research Triangle Park, Durham, USA

[g]Instituto de Investigación en Recursos Cinegéticos (IREC) UCLM-CSIC-JCCM, Ronda de Toledo 12, 13005 Ciudad Real, Spain

[h]Methodology and Scientific Support Unit, European Food Safety Authority (EFSA), Via Carlo Magno 1A, 43126 Parma, Italy

Abstract

Amphibian populations are undergoing a global decline worldwide. Such decline has been attributed to their unique physiology, ecology, and exposure to multiple stressors including chemicals, temperature, and biological hazards such as fungi of the *Batrachochytrium* genus,

[*]Corresponding author: dinicola.matteo@hsr.it. Postal address: Unit of Dermatology and Cosmetology, IRCCS San Raffaele Hospital, Via Olgettina 60, 20132 Milan, Italy.
[§]These authors share first authorship

Author Contributions

Conceptualisation: A.P.T., A.A.T., A.R., E.B.; data curation: A.P.T., A.A.T., A.R., E.B.; writing-original draft preparation: A.P.T.; A.A.T.; A.R., E.B.; M.R.D.N.; writing-review and editing: A.P.T., A.A.T., A.J.W., A.R., E.B., J.LC.M.D., M.R.D.N., N.K., E.C.; supervision: A.R., E.B.; project administration: E.B.; J.L.C.M.D; Conclusion and future perspectives: J.L.C.M.D., M.R.D.N. All authors have read and agreed to the final version of the manuscript.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

viruses such as *Ranavirus,* and habitat reduction. There are limited toxicity data for chemicals available for amphibians and few quantitative structure-activity relationships (QSAR) have been developed and presently available. Such QSARs provide important tools to assess the toxicity of chemicals particularly in a data poor context. QSARs provide important tools to assess the toxicity of chemicals particularly when no toxicological data are available. This manuscript provides a description and validation of a regression-based QSAR model to predict, in a quantitative manner, acute lethal toxicity of aromatic chemicals in tadpoles of the Japanese brown frog (*Rana Japonica)*. QSAR models for acute median lethal concentrations (LC50–12 hours) using the Monte Carlo method were developed. The statistical characteristics of the QSARs were described as average values obtained from five random distributions into training and validation sets. Predictions from the model gave satisfactory results for both the training set (R^2 = 0.661 and RMSE = 0.368) and were even more robust for the validation set (R^2 = 0.965 and RMSE = 0.110). Further development of QSAR models in amphibians, particularly for other life stages and species, are discussed.

Keywords

acute toxicity; *Rana Japonica* tadpole; QSAR; Monte Carlo method; Index of ideality of correlation

1. Introduction

I’m not a diva. I’m a tadpole trying to be a frog. Toni Braxton

The vertebrate class Amphibia, with over 8,420 species, constitutes an important taxonomical group for which all modern amphibians belong to the subclass *Lissamphibia*. They are divided into three orders: *Anura* (frogs, toads, and relatives) with over 7,440 species from 58 families, *Caudata* (salamanders, newts, and relatives) with over 770 species from 9 families and *Gymnophiona* (caecilians and relatives) with over 210 species from 10 families (Frost, 2021). Currently, Amphibian populations are undergoing a global decline in numbers and, over the last five decades, hundreds of species have gone extinct. Such decline has been attributed to their unique physiology, ecology, and exposure to multiple stressors including chemicals, temperature, biological hazards such as fungi of the *Batrachochytrium* genus, viruses such as *Ranavirus,* and habitat reduction (e.g. Wilson and Famini, 1991; Wang et al., 2001; Huang et al., 2003a,b; Roy and Ghosh, 2006; Wang et al., 2019a; Wang et al., 2019b).

Ecotoxicological studies in amphibian species investigating chemical toxicity for substances such as plant protection products and environmental contaminants are still limited since historically more focus has been given to aquatic vertebrates such as fish test species (e.g., rainbow trout, zebrafish) (EFSA PPR, 2018). Data gaps include to the lack of experimental data for different life stages of amphibians as well as lack of regulatory legislation requiring environmental risk assessment (ERA) of chemicals in amphibians. European directives for industrial chemicals and plant protection products require data on aquatic organisms such as insects, fish, daphnia and algae, but not amphibians. However, the European Food Safety Authority (EFSA) has recently published a scientific opinion on the state of the science on

pesticide ERA for amphibians and reptiles to provide a scientific rationale addressing their sensitivity to pesticides, data gaps and to formulate recommendations to further support the inclusion of these taxa in ERA (EFSA PPR, 2018).

In addition, since amphibians have different life stages (i.e., egg, embryo, tadpole, juvenile, and adult), an aquatic phase, and a terrestrial phase; understanding chemical toxicity requires testing such different life stages and consequently considerable economic and experimental efforts. Due to the limited availability of experimental toxicity data in amphibians and the currently limited requirements to address such taxa as ecotoxicological targets, an initial option is to move towards the use of New Approach Methodologies (NAMs) as it has been applied recently to non-target species such as honey bees and collembola (Carnesecchi et al., 2020; Lavado et al., 2021). NAMs include *in silico* models such as quantitative structure-activity relationship (QSAR) models and provide an effective way to predict chemical and toxicological properties based on structural properties of the chemical (ECHA, 2016). The development of QSAR models for tadpoles may be particularly relevant since they are fully aquatic and may be exposed to a range of chemicals throughout their developmental stages making them potentially sensitive as they undergo metamorphosis. However, fewQSARs have been published for frog tadpoles, since experimental values are still rather limited, and have been mostly focused on the prediction of acute toxicity for benzene derivatives in *Rana Japonica*, a limited number of alcohol compounds in *Rana temporaria*, *R. chensinensis,* and for undescribed species (Agrawal et al., 2003; Huang et al., 2004; Jaiswal and Khadikar, 2004; Sahoo et al., 2016; Adhikari and Mishra, 2018; Wang et al., 2019a; Wang et al., 2019b; Wang et al., 2020).

The aim of the present study is to develop a regression-based QSAR model to predict acute toxicity of aromatic chemicals in tadpoles of the Japanese brown frog (*Rana japonica*) using available acute toxicity data and the CORAL software (http://www.insilico.eu/coral). It represents the largest database on available experimental values available in this species. The relevance of this tool for hazard and risk assessment of chemicals and recommendations for future work in this area are discussed to further address chemical toxicity in amphibians.

2. Method

2.1 Database on the acute toxicity of chemicals in *Rana Japonica* tadpoles

Available databases reporting ecotoxicological data on acute median lethal molar concentration in tadpoles of *Rana japonica* were searched for including the Ortiz-Santaliestra et al. (2017) toxicological database on amphibians and reptiles and the US-EPA ECOTOX knowledgebase database, as well as the peer-reviewed literature (Huang et al., 2003a; Wang et al., 2001).

Available compounds in the curated database were distributed into five random splits for four specific subsets from which the last set is used for validation. In contrast, the remaining three sets are used to build the model and optimise parameters. The full procedure which has been shown to provide robust results has been described in detail elsewhere (Toropov et al., 2019, 2020a):

i. Active training set (≈25%) applied for the development of the model and thegeneration of so-called correlation weights. Correlation weights are then used to calculate 2D optimal descriptors for all compounds involved in the modelling process.

ii. Passive training set (≈25%) applied to assess the model robustness for compounds that are independent from those used to build the model. This set is used to assess the improvement of the modelling process in the learning phase.

iii. Calibration set (≈25%) applied to identify when the process of learning reaches it maximum value allowing to extract the general model components providing robust results and identify suitable associated correlation coefficients while reducing the risk of of overfitting.

iv. Validation set (≈25%) providing an independent assessment of the statistical quality of the model using data for substances which were not included in model development and optimisation (Toropov et al., 2017, 2020b; Toropova and Toropov, 2019).

Distributions from the active, passive and calibration sets and the validation set support the assessment of the prediction capacity of a QSAR model (Puzyn et al., 2011). In addition, the Kennard-Stone algorithm (Kennard and Stone, 1969; Morais et al., 2019), duplex algorithm (Snee, 1977), and the response-based division algorithm (Puzyn et al., 2011) provide practical tools to split available data into training and validation sets. In our case, a random distribution was used to split substances, and the non-identity of the splits was assessed.

2.2 Optimal SMILES-based descriptor

The structures associated with the chemicals being modelled in this work using the CORAL models are represented by the simplified molecular input line entry system (SMILES) (Weininger, 1988). The CORAL model is the one-variable correlation between the SMILES-based 2D descriptor and the acute toxicity endpoint (pLC_{50}), according to Equation 1:

$$pLC_{50} = C_0 + C_1 \times DCW(T, N) \quad (1)$$

DCW (Descriptor of Correlation Weights) is a function of the molecular architecture expressed via SMILES, as in Equation 2:

$$DCW(T, N) = \sum CW(S_k) \quad (2)$$

S_k is a SMILES atom, i.e. one symbol (e.g. ‘C’, ‘c’, ‘N’, ‘O’, etc.) or a group of symbols which cannot be examined separately (e.g. ‘Cl’, ‘Br’, etc.). $CW(S_k)$ is the correlation weight of the S_k, i.e. a coefficient which is combined to the value of the descriptor if the corresponding SMILES contains the S_k. The numerical data on the correlation weights are obtained from the Monte Carlo optimisation carried out with the so-called Index of Ideality of Correlation (*IIC*), i.e. a special component of the target function described in

the literature (Toropov and Toropova, 2017; Toropova and Toropov, 2017). The SMILES represent a harmonised format to describe substances for a wide range of *in silico* models, and the structure itself provides a means to calculate molecular descriptors. However, in the case of the CORAL models, SMILES are used directly to extract the information related to the presence of certain encoded molecular features. Such features are represented by atoms, molecular groups, branched structure, presence of rings, and other classical chemical characteristics and have been successfully applied to predict a range of physicochemical and toxicological properties (Toropov and Toropova, 2017; Toropova and Toropov, 2017).

3. Results and Discussion

3.1 Database on acute toxicity of chemicals in *Rana japonica* tadpoles

Available experimental data were reported and extracted as acute lethal concentrations for 50% of *Rana japonica* tadpoles in [mol/L], *i.e.*, negative logarithm of the acute median lethal molar concentrations after 12 h, expressed as 12 h log1/LC50, (pLC_{50}) (Huang et al., 2003a; Wang et al., 2001). The analysis of duplicates confirmed that the same endpoint has been analysed in all available papers and duplicates were excluded from the final database (Supplementary Materials) on acute lethal toxicity data for 58 organic compounds (Table S1). Confirmation of the non-identity of the splits generated is listed in Table S2. Finally, Tables S3-S7 highlights the five splits, with SMILES, experimental and predicted values as well as information regarding the applicability domain of the model and its relevance for each substance.

3.2 QSAR model for predicting acute toxicity of chemicals in *Rana japonica* tadpoles

The Monte Carlo optimisation with and without *IIC* resulted in different models. Table 1 provides the statistical characteristics of the above-mentioned models and the comparison of the data shows that the *IIC*-based optimisation resulted in models with improved statistical quality R^2 (correlation coefficient) and RMSE (root mean squared error expressed by R^2) and particularly while considering the results from the validation set.

The QSAR models for the prediction of acute toxicity in tadpoles of the Japanese brown frog (*Rana Japonica)* (pLC_{50}, mol/L) obtained for the five random splits via the *IIC*-optimisation are the following:

Split 1

$$12h\ pLC_{50} = 1.9257(\pm\ 0.1135) + \quad 0.6528(\pm\ 0.0411) * \mathrm{DCW}(1,15) \tag{3}$$

Split 2

$$12h\ pLC_{50} = \ -0.4164(\pm 0.2701) + \ 0.4524(\pm 0.0274) * \mathrm{DCW}(1,15) \tag{4}$$

Split 3

$$12h\ pLC_{50} = 1.9027(\pm 0.1030) + 0.7199(\pm 0.0393) * \mathrm{DCW}(1,15) \quad (5)$$

Split 4

$$12h\ pLC_{50} = 0.0834(\pm 0.1724) + 0.3844\ (\pm 0.0179) * \mathrm{DCW}(1,15) \quad (6)$$

Split 5

$$12h\ pLC_{50} = 1.7505(\pm 0.0748) + 0.2955(\pm 0.0146) * \mathrm{DCW}(1,15) \quad (7)$$

Table 1 provides a comparison of the prediction results from the QSAR models using optimisation without the *IIC* and models using optimisation with the *IIC*.

The resulting QSAR model using optimisation with the *IIC* provided satisfactory predictions for the validation and training sets compared to the model using optimisation without the *IIC* considering the R^2 on all 58 compounds. However, predictions obtained from the active and passive training sets were slightly less satisfactory (Toropov and Toropova, 2017; Toropova and Toropov, 2017; Toropov et al., 2020; Toropova et al., 2020). The use of optimisation with the IIC is judicious particularly to develop a QSAR model which is not affected by overtraining and is able to predict acute toxicity for substances with no available data. In this way, the model extracts the general components of the algorithm, disregarding those which are closely linked to the training set. The use of multiple sets (active training, passive training, calibration) within the *IIC* strategy is functional here and establishes a dialogue and feed-back loops between the results from the different sets. Finally, the system filters the SMILES attributes with a higher probability to generate a generic model with a broader applicability domain.

Considering the average values for the determination coefficient on the validation sets together with corresponding dispersion provides a measure of uncertainty that supports an assessment of predictions' robustness. In the case of the Monte Carlo optimisation without *IIC*, the average value of the determination coefficient is 0.65 with dispersion 0.10 in contrast to the *IIC*-optimisation with respective values of 0.92 and 0.04. Hence the *IIC* reduces the uncertainty of the prediction for the five computational experiments with splits 1–5.

The average R^2 for the five splits is 0.77 using *IIC*, while without *ICC* the R^2 is 0.72. The QSAR model generated with the third split provided better prediction results while considering the overall statistics ($R^2 = 0.82$) and is concluded to represent the most robust model to be applied for the prediction of acute toixicty in tadpole of *Rana japonica* for data porr compounds.

3.3. Mechanistic interpretation

Table 2 provides the correlation weights ($CW(S_k)$) of the third QSAR model (Eq. 5) including in relation to SMILES attributes (S_k). These values are associated with

quantitative coefficients, thus, providing a score on the relative influence of each parameter. The size of the coefficient is highly informative since it indicates the parameters playing a major role in the determination of acute toxic potency and the sign of the coefficient indicates an increase or decrease of such toxic potency. Table 2 shows that bromine and chlorine atoms increase acute toxicity. Indeed, none of the 10 substances with the lowest acute toxicity potency have chlorine or bromine, while 8 out of 10 of the most toxic substances contain these two atoms. Furthermore, it can also be observed that there are four substances containing three of these atoms, and these four substances are among the five most toxic substances in our dataset. Generally speaking, if the substance contains a single atom of chlorine or bromine, it may have a moderate level of toxicity, unless a nitro group is also present, as discussed below. Overall, the role of chlorine in the determination of toxic potency is in agreement with the conclusions of Huang et al. highlighting that toxicity is associated with chlorine's presence (Huang et al., 2003a). In contrast, fluorine atoms did not impact potency, since this is a relatively small atom with very stable carbon bonds.

The presence of a nitro group was associated with an increase in toxic potency accounted for by the [N+] and [O−] SMILES attributes in Table 2. Indeed, none of the 10 substances with the lowest toxic potency contain chlorine or bromine, while 6 out of 10 of the most toxic substances contain the nitro group. Furthermore, we can also observe that all substances containing two nitro groups have a pLC_{50} superior to 4. Overall, it can also be noted that the co-presence of halogens (bromine and chlorine) and nitro group increases toxic potency.

Conversely, structural features associated with a reduction in toxic potency (negative correlation coefficients) included atoms increasing polarity, such as oxygen and nitrogen in Table 2. Indeed, 9 out of the 10 substances with the lowest toxic potency from the database contain a hydroxy group, while the hydroxy group is present only once in the 10 most toxic substances. This conclusion is also in agreement Huang et al. (Huang et al., 2003a).

The calculation was performed using Eq. 5. Figure 1 contains the graphical representation of the model observed for split #3.

3.4. Comparison with previous QSAR models on *Rana japonica*

Table 4 provides a comparison of the statistical quality of QSAR models from the literature and the model built developed here (Eq.5). The models from the literature applied the quantum mechanics descriptors (Wang et al., 2019a); different physicochemical descriptors, i.e. hydrophobicity, electric property, and molecular size (Huang et al., 2003a) as well, the multiple linear regression based on the extended topochemical atom indices (Roy and Ghosh, 2006). It is to be noted that the models developed here are based on the representation of the molecular structure by SMILES without additional data on physicochemical and quantum mechanics descriptors.

Our QSAR CORAL model is based on 58 substances based on a homogeneous toxicological protocol and dataset. Prediction results and the associated statistics are satisfactory and the tool issimple to used requiring only SMILES without the need for the calculation of chemical descriptors.

4. Discussion and future perspectives

This manuscript describes the development of a regression-based QSAR model predicting acute toxicity in tadpoles of *Rana Japonica* for a range of aromatic compounds with satisfactory results based on the *IIC* metric, particularly for the validation set. There are several interesting points related to the present study. (1) There are only few QSAR models are available for amphibians; (2) this study is based on a relatively large data set of substances tested for the same species; (3) it is based on a quite simple approach which only requires SMILES format, without the need for calculating chemical descriptors; (4) it identifies a number of chemical features which can be used to characterise acute toxicity in tadpoles; (5) such chemical features can be used pro-actively prioritise substances with high toxic potency and compared to substances associated with low toxic potency.

In terms of ERA, the future development of QSAR models also requires consideration of the taxonomic framework of “true frogs” and is important to pinpoint which species can be considered as representative of the whole genus and sub-genus for different geographical locations. In this context, the genus and sub-genus *Rana* is considered the lineage of “true frogs” (family *Ranidae*) and associated with 106 associated species that are present in Europe, Asia and the Americas (Najibzadeh et al., 2017). So far, 106 and 54 species have been described depending on the different taxonomic considerations of the subspecific levels amongst the different (AmphibiaWeb, 2021; Frost, 2021; Najibzadeh et al., 2017). Yuan et al., (2016) carried out a comprehensive phylogenetic assessment of the taxon, considering 101 species distributed in Eurasia and the Americas and divided the subgenus *Rana* into a number of clades and subclades: two in East Asia, one in Europe and Central Asia (see Figure 2 in Yuan et al., 2016). The Eurasian species of the subgenus *Rana* “brown frogs” are phylogenetically related, morphologically conserved and characterised by a dorsal colour with different shades of brown, the presence of evident dorsolateral folds and a dark temporal mask (Boulenger, 1920; Liu and Hu, 1961; Yuan et al., 2016). This implies that the identification on a morphological basis is often difficult and the description of new species nowadays is based on molecular features using nuclear and mitochondrial DNA (Yuan et al., 2016; Zhao et al., 2017). In this context, the Japanese brown frog *Rana japonica* (Boulenger, 1920) was originally described as *Rana temporaria var. japonica* (Gunther, 1859) and is distributed in Japan (Honshu, Kyushu and Shikoku islands and Tanegashima Group) (Amphibian species of the world 6.1, 2021). The taxon belongs to the aforementioned Eurasian clade which also includes the European common frog *Rana temporaria* and is widespread from northern to southern Europe (Yuan et al., 2016). A focus on the phylogenetic relationship between the two species is shown in Figure 2.

In addition to the similar morphology common to all “brown frogs”, *Rana japonica and Rana temporaria* are both taxa that live in the temperate belt of the northern hemisphere occupying assimilable environmental typologies characterised by the presence of four distinct seasons and also share an explosive breeding modality, which takes place in late winter / early spring (Di Nicola et al., 2021; Lanza et al., 2007; Matsushima and Kawata, 2005). Hence, available chemical toxicity data for *Rana japonica* is well suited for the development of regression-based QSAR models to address chemical toxicity in anuran amphibians including the European brown frog (*Rana temporaria*). However, developments

of regression-based QSAR models for anuran amphibian species is warranted to predict acute toxicity in different life stages of *Rana japonica* and *Rana temporaria* as well as North American species such as the Northern leopard frog (*Rana pipiens)* and the American bullfrog (*Lithobates catesbeianus*) within their aquatic and terrestrial phase (egg, embryo, tadpole, juvenile and adult). In addition, the development of similar QSAR models for the African clawed frog (*Xenopus laevis*), as an OECD amphibian test species with a strictly speaking aquatic lifestyle, can provide another important tool for risk assessors for predicting chemical toxicity in amphibians particularly for plant protection products and environmental contaminants.

Two major data gaps for hazard and risk assessment in amphibians include the lack of chronic toxicity in anuran amphibians and the lack of toxicity data and QSAR models in *Caudata* (salamanders and newts) as well as *Gymnophiona* (caecilians and relatives). Moreover, since very limited kinetic information is available for anuran amphibians, options to further investigate fate and bioaccumulation in anuran amphibians is compromised. Since chemical toxicity data in fish are more readily available, an option is to use such data for cross-species read-across as well as data collection and generation of kinetic data, as well as quantitative physiological data and life cycle data for amphibians, would allow for the development of physiologically-based kinetic models and the derivation of bioactive concentrations on an internal basis for acute and chronic endpoints. It would also allow for the calibration and validation of dynamic energy budget models to investigate the impact of chemicals at the individual and population level (Grech et al., 2016; Baas et al., 2018).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Few QSAR models are available for the prediction of chemical toxicity in amphibians
- QSAR models have been developed here to predict acute lethal toxicity of aromatic chemicals in *Rana japonica*
- Satisfactory prediction results for the training set and robust results for validation set
- QSAR model development for other life stages and amphibian species are proposed

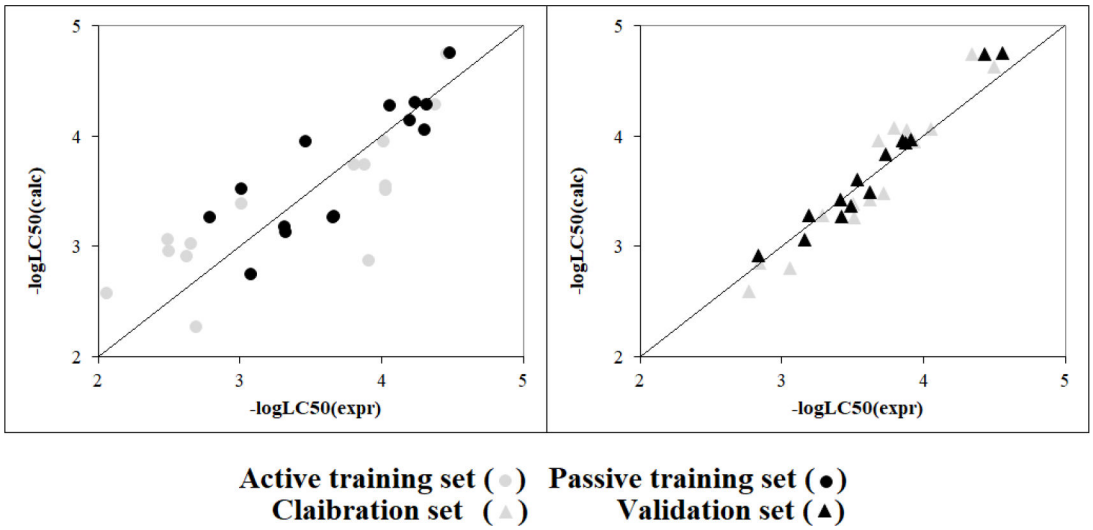

Figure 1.

Models observed for split #3 from Monte Carlo optimisation using *IIC*.

X axis: -LogLC50 (calc): calculated negative logarithm of the acute median lethal molar concentrations after 12 h, in *Rana Japonica* tadpoles; Y axis: -LogLC50 (expr): experimental negative logarithm of the acute median lethal molar concentrations after 12 h, in *Rana Japonica* tadpoles.

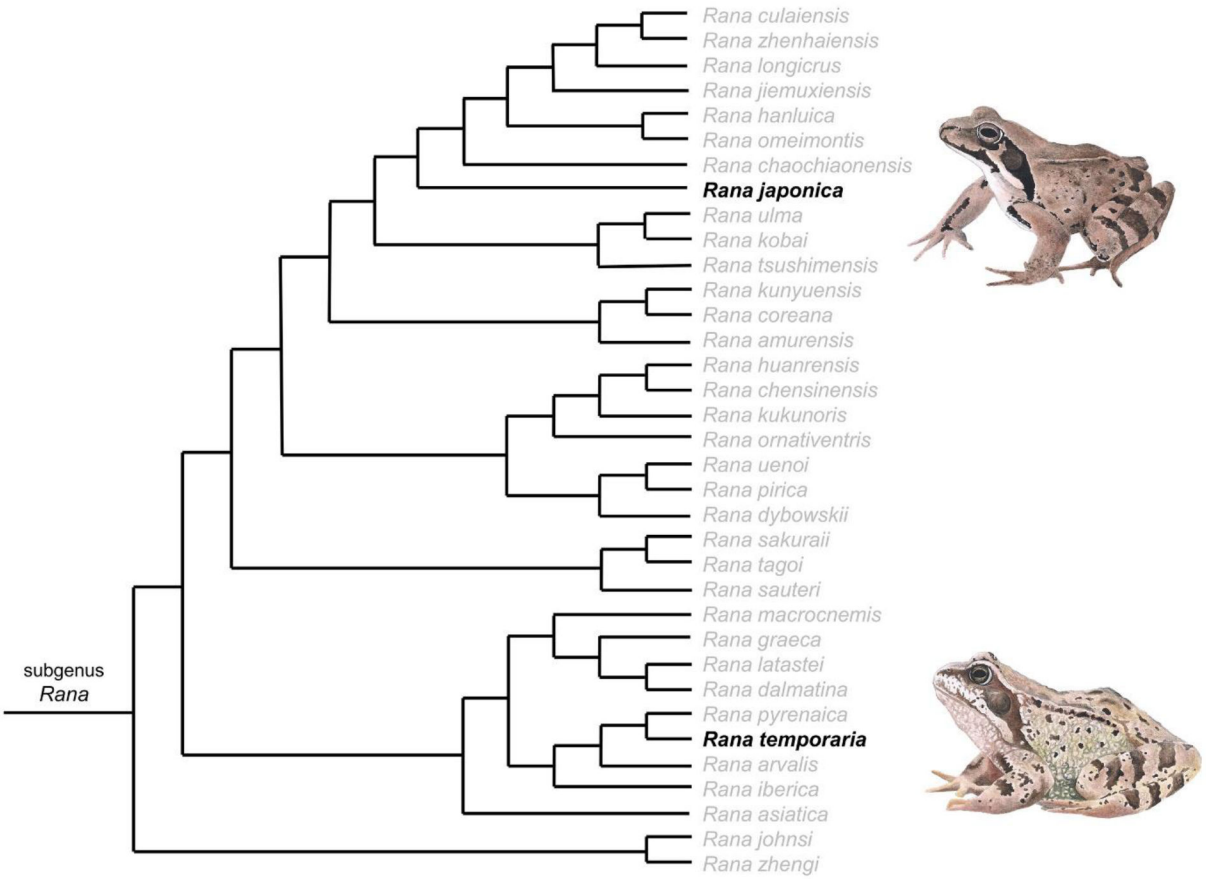

Figure 2.

Simplified tree that shows phylogenetic relationship between *Rana japonica* and *Rana temporaria*. Modified from Yuan et al. (2016).

Table 1.

Statistical characteristics of the QSAR model using five random splits

Split	Set	*n*	R^2	*CCC*	*IIC*	Q^2	Q^2_{F1}	Q^2_{F2}	Q^2_{F3}	$\langle R_m^2 \rangle$	*RMSE*
Optimisation without *IIC*											
1	A*	14	0.807	0.893	0.499	0.721					0.284
	P	15	0.810	0.891	0.594	0.748					0.335
	C	15	0.171	0.318	0.225	0.0	0.0	0.0	0.224	0.071	0.612
	V	14	0.822								0.495
2	A	14	0.753	0.859	0.651	0.673					0.349
	P	15	0.902	0.722	0.397	0.876					0.577
	C	15	0.754	0.574	0.160	0.641	0.108	0.0	0.756	0.569	0.384
	V	14	0.558								0.308
3	A	15	0.699	0.823	0.558	0.595					0.443
	P	14	0.736	0.844	0.748	0.662					0.325
	C	15	0.816	0.874	0.4688	0.759	0.759	0.7491	0.880	0.738	0.250
	V	14	0.583								0.366
4	A	15	0.928	0.9629	0.843	0.902					0.166
	P	15	0.708	0.838	0.688	0.609					0.432
	C	14	0.833	0.899	0.568	0.770	0.773	0.7733	0.871	0.736	0.247
	V	14	0.709								0.356
5	A	14	0.8544	0.922	0.693	0.822					0.246
	P	14	0.8145	0.867	0.5477	0.741					0.358
	C	15	0.5607	0.732	0.3723	0.368	0.381	0.365	0.688	0.417	0.401
	V	15	0.5971								0.346
Optimisation with *IIC* (Eq. 3 – Eq.7).											
1	A*	14	0.603	0.753	0.777	0.476					0.407
	P	15	0.458	0.671	0.578	0.278					0.566
	C	15	0.942	0.967	0.967	0.923	0.933	0.928	0.961	0.860	0.136
	V	14	0.876								0.215
2	A	14	0.586	0.739	0.765	0.462					0.453
	P	15	0.839	0.893	0.474	0.805					0.379
	C	15	0.789	0.840	0.888	0.744	0.772	0.545	0.938	0.567	0.194
	V	14	0.869								0.177
3	A	15	0.667	0.800	0.715	0.564					0.466
	P	14	0.709	0.840	0.557	0.636					0.327
	C	15	0.925	0.932	0.902	0.900	0.826	0.819	0.913	0.693	0.212
	V	14	0.962								0.140
4	A	15	0.623	0.767	0.690	0.528					0.381
	P	15	0.573	0.749	0.572	0.456					0.511

Split	Set	*n*	R^2	*CCC*	*IIC*	Q^2	Q^2_{F1}	Q^2_{F2}	Q^2_{F3}	$\langle R_m^2 \rangle$	*RMSE*
	C	14	0.969	0.981	0.981	0.9554	0.965	0.965	0.980	0.900	0.097
	V	14	0.965								0.110
5	A	14	0.656	0.792	0.607	0.559					0.378
	P	14	0.624	0.699	0.337	0.426					0.502
	C	15	0.885	0.900	0.938	0.827	0.832	0.828	0.915	0.734	0.209
	V	15	0.918								0.178

*) A = active training set; P = passive training set; C = calibration set; V = validation set; n = number of compounds in each set; R^2 = correlation coefficient; RMSE = root mean squared error; Q^2 = cross validated R^2; CCC = concordance correlation coefficient (Lin, 1992); *IIC* = index of ideality of correlation (Toropov and Toropova, 2017); Q^2_{F1}; Q^2_{F2}; Q^2_{F3} (Chirico and Gramatica, 2011); $\langle R_m^2 \rangle$ (Roy and Kar, 2014) are criteria of the predictive potential suggested in the literature.

Table 2.

Correlation weights ($CW(S_k)$) obtained by Monte Carlo optimisation for the third split (Eq. 5).

S_k	$CW(S_k)$
(.....	−0.0813
1.....	0.8421
2.....	1.0824
=.....	−0.2139
C.....	0.4530
F.....	−0.2871
Br.....	1.1052
Cl.....	1.0972
N.....	0.0
O.....	0.1401
S.....	0.0
[N+].....	1.1961
[O−].....	0.1351
c.....	−0.1438

Table 3.

Experimental and predicted acute toxicity in tadpoles of the brown Japanese frog (*Rana japonica*) for the third split.

Set	CAS	SMILES	*DCW (1,15)*	$pLC_{50\ Expr}$	$pLC_{50\ Calc}$	Expr-Calc
C	6627-55-0	Cc1cc(Br)c(O)cc1	2.1944	3.7200	3.4823	0.2377
A	831-82-3	Oc2ccc(Oc1ccccc1)cc2	2.2405	4.0300	3.5155	0.5145
V	87-61-6	Clc1cccc(Cl)c1Cl	3.9502	4.4310	4.7462	−0.3152
C	120-82-1	Clc1cc(Cl)c(Cl)cc1	3.7876	4.5000	4.6292	−0.1292
V	56961-77-4	Clc1cccc(Br)c1Cl	3.9582	4.5600	4.7520	−0.1920
P	19393-92-1	Clc1cccc(Cl)c1Br	3.9582	4.4810	4.7520	−0.2710
C	541-73-1	Clc1cccc(Cl)c1	2.8530	3.6790	3.9564	−0.2774
V	106-46-7	Clc1ccc(Cl)cc1	2.8530	3.8500	3.9564	−0.1064
C	95-50-1	Clc1ccccc1Cl	3.0155	3.7900	4.0734	−0.2834
V	108-90-7	Clc1ccccc1	1.9183	3.1950	3.2836	−0.0886
C	108-95-2	Oc1ccccc1	0.9612	2.7690	2.5947	0.1743
A	95-57-8	Oc1ccccc1Cl	2.0584	3.0110	3.3845	−0.3735
P	106-41-2	Oc1ccc(Br)cc1	1.9039	3.6640	3.2732	0.3908
V	106-48-9	Oc1ccc(Cl)cc1	1.8959	3.4210	3.2675	0.1535
A	371-41-5	Fc1ccc(O)cc1	0.5116	2.6930	2.2710	0.4220
A	90-05-1	Oc1ccccc1OC	1.5543	2.6540	3.0216	−0.3676
V	95-48-7	Cc1ccccc1O	1.4142	2.8370	2.9207	−0.0837
A	150-76-5	Oc1ccc(OC)cc1	1.3918	2.6240	2.9046	−0.2806
C	106-44-5	Cc1ccc(O)cc1	1.2517	3.0570	2.8037	0.2533
A	98-54-4	CC(C)(C)c1ccc(O)cc1	2.2856	4.0330	3.5480	0.4850
P	576-26-1	Cc1cccc(C)c1O	1.7047	3.3240	3.1298	0.1942
A	90-15-3	Oc2cccc1ccccc12	2.5506	3.8070	3.7388	0.0682
A	135-19-3	Oc1ccc2ccccc2c1	2.5506	3.8860	3.7388	0.1472
V	120-83-2	Clc1cc(Cl)c(O)cc1	2.8305	3.8730	3.9403	−0.0673
A	108-46-3	Oc1cccc(O)c1	0.9388	2.0660	2.5785	−0.5125
P	80-5-7	CC(C)(c1ccc(O)cc1)c2ccc(O)cc2	3.1118	4.2010	4.1427	0.0583
V	612-00-0	CC(c1ccccc1)c2ccccc2	2.8663	3.9140	3.9660	−0.0520
V	554-00-7	Clc1cc(Cl)c(N)cc1	2.6904	3.7320	3.8394	−0.1074
V	74-11-3	OC(=O)c1ccc(Cl)cc1	2.1125	3.4170	3.4234	−0.0064
C	586-76-5	OC(=O)c1ccc(Br)cc1	2.1206	3.6250	3.4292	0.1958
C	69-72-7	OC(=O)c1ccccc1O	1.3180	2.8400	2.8515	−0.0115
P	321-14-2	Oc1ccc(Cl)cc1C(=O)O	2.2526	3.0110	3.5243	−0.5133
P	123-08-0	O=Cc1ccc(O)cc1	1.1779	3.0800	2.7506	0.3294
C	98-95-3	[O−][N+](=O)c1ccccc1	1.9159	3.2860	3.2819	0.0041
V	88-72-2	Cc1ccccc1[N+](=O)[O−]	2.3689	3.5300	3.6080	−0.0780
V	99-99-0	Cc1ccc(cc1)[N+](=O)[O−]	2.2063	3.6240	3.4910	0.1330

Set	CAS	SMILES	*DCW (1,15)*	$pLC_{50\ Expr}$	$pLC_{50\ Calc}$	Expr-Calc
C	88-75-5	O=[N+]([O−])c1ccccc1O	2.0560	3.5020	3.3827	0.1193
C	554-84-7	O=[N+]([O−])c1cccc(O)c1	1.8934	3.5100	3.2657	0.2443
P	100-02-7	O=[N+]([O−])c1ccc(O)cc1	1.8934	3.6570	3.2657	0.3913
C	100-00-5	O=[N+]([O−])c1ccc(Cl)cc1	2.8505	3.9340	3.9547	−0.0207
A	100-11-8	O=[N+]([O−])c1ccc(CBr)cc1	3.3116	4.3830	4.2866	0.0964
P	100-14-1	O=[N+]([O−])c1ccc(CCl)cc1	3.3035	4.3210	4.2808	0.0402
C	89-64-5	Oc1ccc(Cl)cc1[N+]([O−])=O	2.9906	3.8820	4.0555	−0.1735
V	601-89-8	O=[N+]([O−])c1c(O)cccc1O	2.0335	3.4920	3.3666	0.1254
P	6283-25-6	Nc1cc(ccc1Cl)[N+]([O−])=O	2.8505	3.4660	3.9547	−0.4887
P	776-34-1	[O−][N+](=O)c1ccc(N)c2ccccc12	3.3427	4.2360	4.3090	−0.0730
C	528-29-0	O=[N+]([O−])c1ccccc1[N+]([O−])=O	3.0107	4.0500	4.0700	−0.0200
A	99-65-0	O=[N+]([O−])c1cccc(c1)[N+]([O−])=O	2.8481	4.0150	3.9529	0.0621
P	121-14-2	Cc1ccc(cc1[N+](=O)[O−])[N+]([O−])=O	3.3011	4.0610	4.2790	−0.2180
P	51-28-5	O=[N+]([O−])c1cc(ccc1O)[N+]([O−])=O	2.9882	4.3060	4.0538	0.2522
A	584-48-5	O=[N+]([O−])c1cc(ccc1Br)[N+]([O−])=O	3.9534	4.4610	4.7485	−0.2875
C	97-00-7	O=[N+]([O−])c1cc(ccc1Cl)[N+]([O−])=O	3.9453	4.3420	4.7428	−0.4008
A	90-02-8	O=Cc1ccccc1O	1.3404	3.9140	2.8676	1.0464
P	119-36-8	Oc1ccccc1C(=O)OC	1.7710	3.3150	3.1776	0.1374
V	99-76-3	Oc1ccc(cc1)C(=O)OC	1.6084	3.1600	3.0605	0.0995
P	945-51-7	O=S(c1ccccc1)c2ccccc2	1.8865	2.7900	3.2607	−0.4707
A	99-93-4	O=C(C)c1ccc(O)cc1	1.4683	2.5030	2.9597	−0.4567
A	156-38-7	Oc1ccc(CC(=O)O)cc1	1.6084	2.4970	3.0605	−0.5635

*)A = active training set; P = passive training set; C = calibration set; V = validation set

Table 4.

Comparison of the statistical performance of the QSAR models for acute toxicity in tadpoles of the brown Japanese frog (*Rana japonica*)

N	R^2(training)	RMSE(training)	N	R^2(validation)	RMSE(validation)	Reference
9	0.930	0.220	-	-	-	Wang et al., 2019a
51	0.834–0.914	0.243– 0.175	-	-	-	Huang et al., 2003a
51	0.915	0.183	-	-	-	Roy and Ghosh, 2006
44	0.722	0.330	14	0.965	0.110	Eq.5