The use of High Accuracy QSAR models in high level testing.

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Replacing Experimentation

Introduction

Micro/Mesocosm systems have added much to the existing knowledge of ecologists facilitating a better understanding of the interactions, fate and effects within ecosystems. Moreover, enclosed model ecosystems are becoming a major research tool for soil/sediment ecologists because of the high degree of control of test parameters (light, heat, pH, hygrometry…) that these systems allow compared to conventional methods. Despite being an efficient methodology to mimic field experiments, micro and mesocosms are associated with several drawbacks, for instance the identification of correct dose levels which would result in effects needed to determine the interactions which would be found in natural ecosystems and the reliable extrapolation of laboratory based ecosystems to the field experiments[1]. *In-silico* alternatives including Quantitative Structure Activity Relationships (QSARs) have been proposed in the recent years in response to the EU initiatives to minimize animal testing by replacing them with suitable and reliable approaches. QSARs are based on the principle that similar chemical structures can lead to similar biological activities and are commonly applied in the ecotoxicological risk assessment to evaluate the fate of chemicals in the environment. Recently, KREATiS developed a series of QSAR models called 'HA-QSARs' included in it's iSafeRat Toolbox[2] which in comparison to standard QSARs offers predicted values reliable enough to replace experimental studies and validated following the criteria specified in regulatory guidelines. The aim of this poster is to provide an introduction to the methodology for development and validation of HA-QSARs and briefly present ways in which such models of high precision can be fruitfully used to provide input data to help overcome the some of the above limitations in experimentation and achieve the best possible quality studies.

Existing issues with experimentation

a) Since the environmental impact may vary depending on the concentration of the substance used, multiple replicates have to be included in the design at different concentrations. However, the larger the volume used in the study, the fewer the reps and concentrations.

b) If the physicochemical properties of a substance are not known beforehand, serious errors in predicted fate properties and in maintaining constant concentrations during the testing period (for instance volatility or adsorption issues) can be made, invalidating the experiment.

c) Since an enclosed model ecosystem is expected to mimic real field situations, it would be helpful to have a understanding of chronic effects concentrations on single species prior to the experiement so that the micro/mesocosm can account specifically for interactions between populations and trophic levels.

Use of HA-QSARs for high quality testing

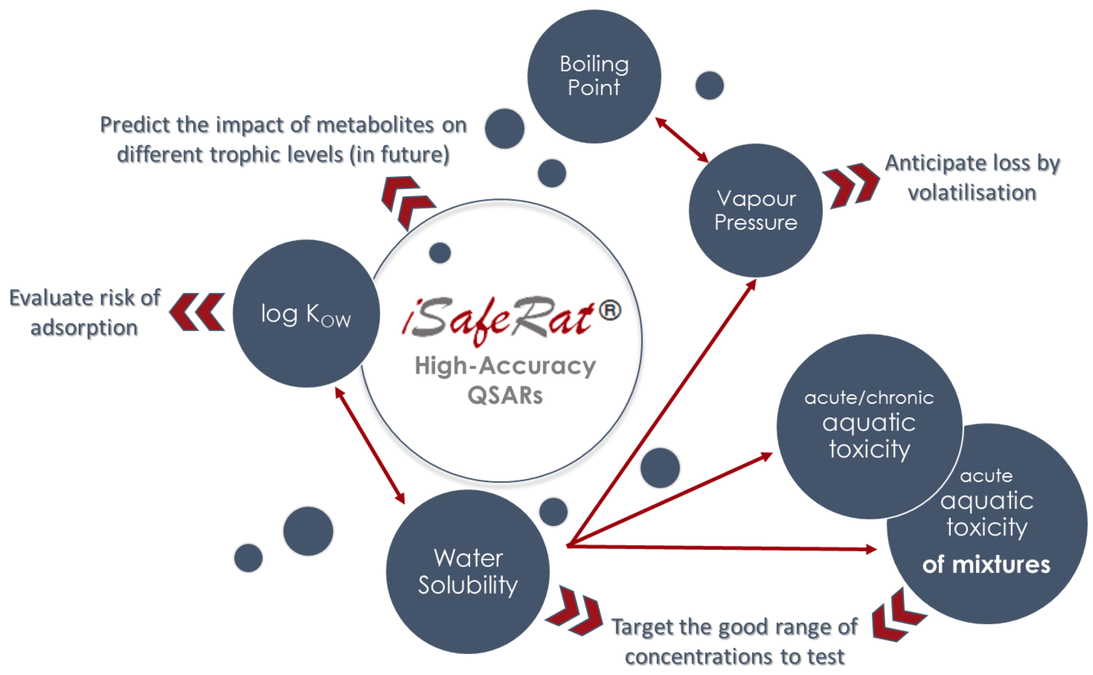

Figure 1: Usefulness of iSafeRat® HA-QSARs for high quality testing

KREATiS' work is based on holistic approach (Figure 1) where every endpoint is interlinked, and physicochemical properties are determined using thermodynamic equilibria. This method is a powerful tool to validate QSAR predictions and experimental results between each other.

HA-QSAR models can precisely determine the physicochemical and ecotoxicological properties (acute and chronic toxicity) of MOA1/MOA2 substances using thermodynamic principles [3]. For other modes of action, HA-QSARs can be prepared on a case by case basis, thus allowing adaptations to specific experimental setup requirements. For instance, as shown in the figure, results derived with different endpoints can be useful to evaluate various parameters including the adsorption, volatility issues as well as getting a better estimation of the concentration range optimal for the testing purpose. In future we aim to develop a predictive model to take into account the metabolites which could be helpful to deduce indirect ecotoxicological effects at different trophic levels within a complex experimental system including mesocosms.

How are HA-QSARs developed?

Step 1: Data collection

Experimental data on physicochemical and ecotoxicological endpoints is collected for different chemical substances from various literature resources including publicly available databases, publications and reports.

Substance	Property 1	Property 2	Property 3	Property 4	Property 5
Substance 1	0.11	1.32	5.54	120.87	0.87
Substance 2	0.43	3.44	6.50	133.32	0.43
Substance 3	0.23	3.22	7.55	187.89	0.66
Substance 4	0.34	1.00	7.68	180.99	0.99
Substance 5	0.55	5.66	8.77	199.00	1.01

Step 2: Data validation

The collected data then undergoes rigorous data check to ensure the measured endpoint values were derived using appropriate test methods and under suitable testing conditions. For instance, in the table the cells are highlighted in different colours to indicate the quality of measured values (green=reliable; yellow=less accurate; red=not reliable).

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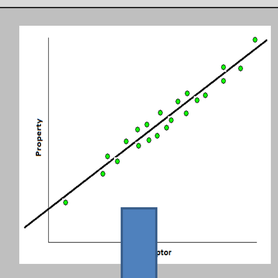
Substance 4	0.34	1.00	7.68	180.99	0.99
Substance 5	0.55	5.66	8.77	199.00	1.01

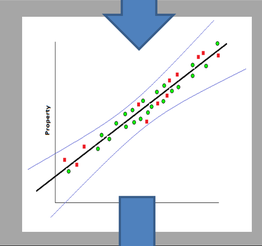
Step 3: Model development

Depending upon the relationship between the chosen set of descriptors and the endpoint values, an appropriate modelling strategy is finalised, for instance Simple or Multiple Linear regression.



Step 4: Model validation

Once the approach is finalized, model is generated and then can be used to predict the endpoint values for an external test set. HA-QSARs at KREATiS are validated as per the five OECD principles for model validation.



Step 5: Cascade approach validation

Since all the physicochemical and ecotoxicological endpoints forming the cascade approach are interlinked, data gaps are filled and the predicted value for each endpoint is compared with validated experimental data, if available. This step facilitates an additional level of validation to ensure that the cascade approach works.

Log Kow				Water Solubility (mg/L)				LC50 Fish - 96h (mg/L)			
Measured	iSafeRat® v1.1	KOWWIN v1.68	QSAR Toolbox v3.0	Measured	iSafeRat® v1.1	WSKOWWIN v1.42	QSAR Toolbox v3.0	Measured	iSafeRat® v1.1	ECOSAR v1.11	QSAR Toolbox v3.0
3.39	3.45	2.80	2.88	38.50	34.71	49.76	32.30	4.26	1.85	11.41	10.20
5.13	5.27	4.77	4.95	1.90	2.49	6.90	2.63	1.01	0.35	0.50	2.50
3.3	3.16	3.38	3.26	456.00	337.36	434.50	415.00	18.90	15.83	7.38	2.43
4.3	4.43	5.14	No RA	20.00	24.56	5.26	NN issue	1.10	1.91	1.37	1.37
4.7	4.62	5.68	4.50	14.10	16.10	1.53	18.00	1.80	1.43	1.21	2.12
2.60	2.38	3.33	3.13	2540.00	3758.48	371.70	710.00	62.00	68.61	8.07	4.09
4.2	4.25	4.93	NN issue	34.10	39.58	7.84	No RA	2.30	2.71	0.40	NN issue
2.67	2.81	2.64	NN issue	193.00	225.29	206.10	NN issue	4.99	7.14	15.40	13.80
4.01	4.03	3.76	NN issue	7.50	8.17	27.35	NN issue	3.00	1.70	3.35	3.49
3.40	3.31	3.17	3.92	31.00	39.92	142.10	134.00	6.08	6.25	9.39	5.68
1.61	1.54	1.67	NN issue	6130.00	13381.78	6484.00	NN issue	162.00	161.52	193.85	5.74
2.75	2.78	3.06	No RA	440.00	730.07	173.40	No RA	14.00	13.07	5.02	2.90
2.62	2.31	2.53	NN issue	1960.00	2157.42	1610.00	NN issue	43.00	44.64	38.80	17.30
1.52	2.08	1.88	NN issue	3110.00	2839.55	5800.00	NN issue	91.70	50.15	106.61	27.10
3.07	2.98	2.71	No RA	450.00	362.87	455.60	No RA	5.50	4.24	3.12	36.80
Avg. Err	*0.14*	*0.40*	*1.56*	*Avg. folds*	*1.31*	*3.16*	*2.14*	*Avg. folds*	*1.47*	*2.48*	*5.78*

Conclusions

The KREATiS HA-QSARs were evaluated as a potential cheap rapid and helpful tool to accompany high tier experimental testing strategy. The results from HA-QSARs can be used to facilitate greater understanding of the interactions at different trophic levels within complex experimental systems and to help determine concentration levels to be used. Research is ongoing into future HA-QSARs accounting for the ecotoxicological effects of metabolites.

References

[1] C. Kampichler, A. Bruckner, E. Kandeler, Use of enclosed model ecosystems in soil ecology: a bias towards laboratory research, Soil Biology and Biochemistry, Volume 33, Issue 3, March 2001, Pages 269-275

[2] iSafeRat® – in Silico Algorithms For Environmental Risk And Toxicity version 1.1

[3] ECETOC Technical Report No. 120, 2013. Relationships of quantitative structure-activity to comparative toxicity of selected phenols in the Pimephales promelas and Tetrahymena pyriformis test systems. ECETOC Technical Report No. 120. European Centre for Ecotoxicology and Toxicology of Chemicals.

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