Replacing Experimentation

A unique QSAR model to determine microorganism toxicity in activated sewage sludge.

Paul THOMAS, Jennifer OSES, Pascal BICHEREL

KREATiS, Knowledge & Research in Environment And Toxicology in Silico

KREATiS SAS, 23, rue du Creuzat, ZAC St Hubert, 38080 L'ISLE D'ABEAU, FRANCE

www.kreatis.eu

Introduction

Toxicity of microorganisms, usually following the Activated Sludge Respiration Inhibition Test (ASRIT) – OECD guideline 209[1], is a required endpoint in fulfilment of Annex VIII in REACH dossiers i.e. substances produced in quantities >10 TPA. Physicochemical properties (such as adsorption to sludge or volatility) as well as the lack of obligation for analytical quantification makes it difficult to perform this test for some substances and interpretation can be ambiguous. In order to replace this kind of experimentation a High-Accuracy EC50 QSAR model has been developed. This work has been carried out as a part of the DAMIER project, a French funded project for the development and the use of High Accuracy QSAR models for REACH compliance. Work on this model was initiated to complete the set of KREATiS acute aquatic ecotoxicity QSARs. During the project other endpoints and studies were also considered for modelling, notably the EC10 and NOEC values for the 30 minute and 3 hour ASRIT test and the 16 h *Pseudomonas putida* growth inhibition test. The available datasets for these ASRIT endpoints and the *P. putida* study were not found to be of satisfactory quality to allow the production of a suitable QSAR and work therefore focussed on a high accuracy 3h ASRIT EC50 QSAR.

Methodology

Data was collected from major data banks, particularly the ECHA disseminated database, OECD SIARS and EU existing substances Risk Assessments. The dataset was preliminary divided up according to the Mode of Action of the structures retrieved. Data was then curated by separating out different test types (ASRIT, *P. putida* growth test, read-across from other molecules, use of biodegradation study test substance concentrations which do not constitute a concentration response relationship, excluding greater than values that would not allow precise quantification of effects value…). Remaining studies were individually evaluated for their potential usefulness.

Results

Of the 1174 molecules included in the data search, only 377 molecules provided endpoint data that were appropriate for risk assessment (NOEC, LOEC, EC_{10}, EC_{20} ou EC_{50}) related to the ASRIT. Of these, 258 substances contained values potentially usable in the development of a 3h EC50 QSAR. Many studies indicated a range or maximum concentrations tested where no inhibition occurred over the test. Of these, only 30 molecules were as MoA 1. 7 were assessed as poor quality.

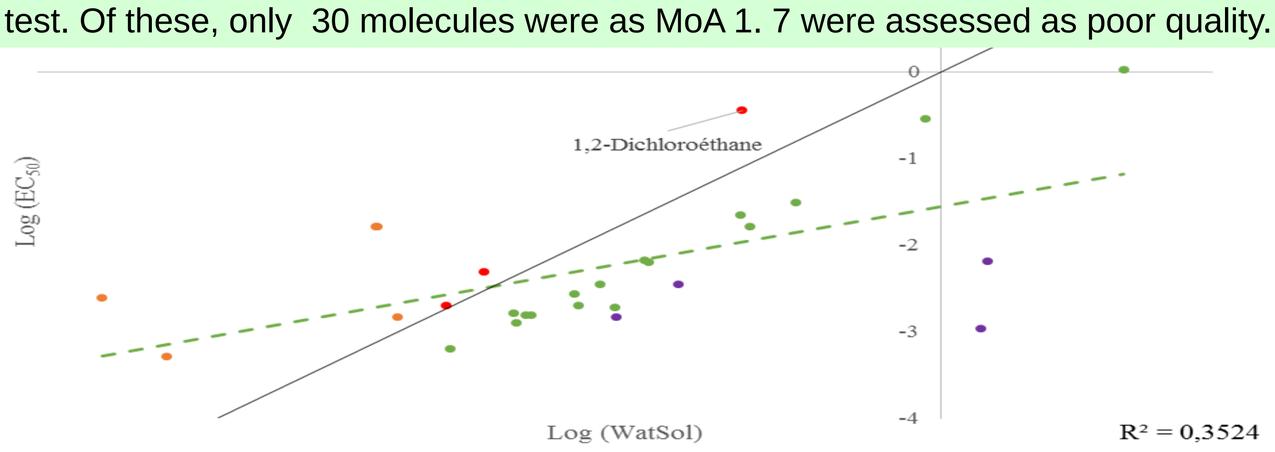

Figure 1: Initial relationship between the concentration of the substance test causing 50% inhibition of respiration to activated sludge (EC_{50} in mol/L) and its water solubility (WatSol in mol/L), (n = 27). The black line corresponds to the limit of solubility of the substances in water (above which the toxicity of substances is greater than the water solubility limit). The orange points correspond to molecules with an EC50 > solubility. The mauve points correspond to studies which did not follow OECD 209. Red points correspond to studies of poor experimental quality which were excluded from the regression. The green line is a linear regression based on 27 substances.

Several points > solubility were excluded from the regression but included in the graph to indicate the point where equilibrium is not reached within the 3 hour timeline and toxicity is > solubility limit. This appears to occur at a surprisingly low hydrophobicity level, around log Kow of 3 for MoA 1 susbtances. The remaining points were used to obtain a regression and the R^2 considered sufficiently high for the model to be labelled a HA-QSAR for the EC50.

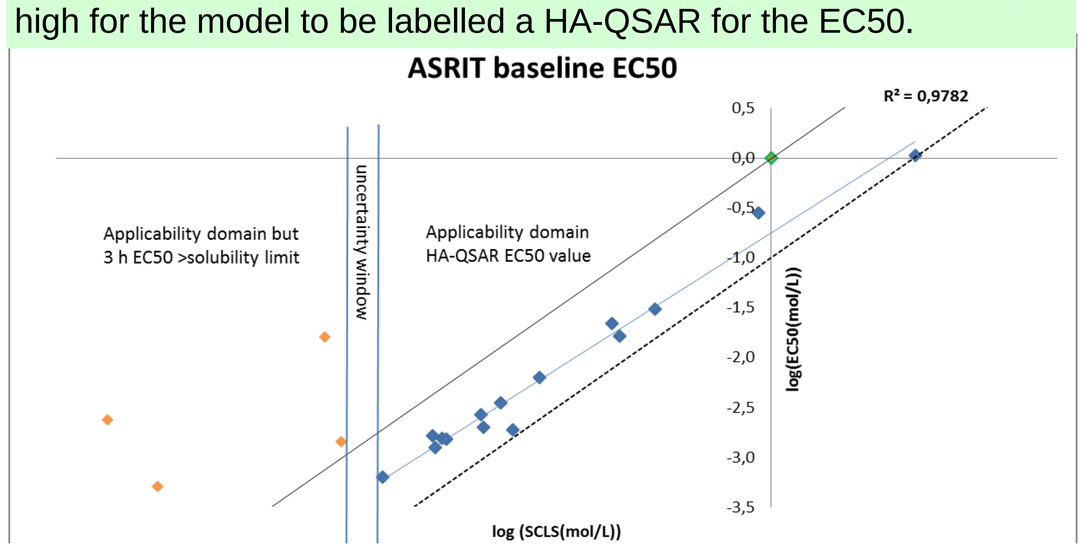

Figure 2: Final relationship between the concentration of the test substance causing 50% inhibition of respiration to activated sludge (EC_{50} in mol/L) and their sub-cooled liquid solubility.

Discussion

Despite the (rather surprisingly) limited number of quality data available for the construction of this acute model, the final R^2 and numerous other statistical hurdles necessary to demonstrate accuracy within the applicability domain were achieved for the ASRIT EC50 QSAR. The model meets the five OECD principles (OECD, 2004) and has been validated to provide accurate EC50 values for non-polar narcotic substances. The algorithm is based on a simple linear relationship which links toxicity to activated sludge microorganisms to sub-cooled liquid solubility. It is based on the concept that chemical activity expressed through water solubility can explain aquatic toxicity as has previously been shown for fish, invertebrates and algae. This is the first model in ecotoxicity able to predict such an endpoint for regulation known to the authors. Attempts to determine quality relationships using ASRIT NOEC/EC10 data and the *Pseudomonas* growth inhibition test were not conclusive. KREATiS will pursue QSAR ASRIT models for other MoAs.

Summary

Determination of bacterial inhibition in WWTPs is an obligation for Annex VIII dossiers under REACH. As part of the DAMIER project, KREATiS has prepared an ASRIT HA-QSAR using available data. Only limited quality data were retrieved but these were sufficient to provide a relationship meeting the OECD principles within the applicability domain.

References

[1]**OECD** (2010). Test No. 209 : Activated Sludge, Respiration inhibition test (Carbon and Ammonium oxidation). OECD Guidelines for the Testing of Chemicals, Section 2, Editions OECD, Paris.

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