The utility of QSARs in predicting acute fish toxicity of pesticide metabolites: a retrospective validation approach

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Introduction

- Registrants are required to determine if pesticide metabolites are harmful to animals and the environment (Regulation (EC) No. 1107/2009).
- Assessments are triggered for the aquatic environment when a metabolite is detected at levels >10% of applied amount of parent substance in a water sediment system.
- The toxicity of metabolites is assessed in standard algal, daphnid and fish tests.
- The number of metabolites requiring assessment can be considerable, therefore this is an area where many vertebrates could be used.
- The EFSA aquatic guidance document (EFSA 2013) suggests that alternative methods could be used to confirm that metabolites are of low risk.
- One of the recommended non-testing approaches to predict toxicity endpoints are Quantitative Structure Activity Relationship (QSAR) models, which are becoming more commonly used in a regulatory context (e.g. under REACH).
- However scientific evidence is needed, specifically for plant protection product metabolites, to confirm such a method is "fit for purpose".
- **AIM: To examine the potential for QSARs to be used in the prediction of acute fish toxicity of pesticide metabolites.**
- If this approach is viable and gains acceptance, it could reduce the numbers of vertebrate animals used significantly.
- Non-testing approaches also address some of the practical issues associated with metabolite testing (e.g. synthesis, solubility and stability).

Materials and methods

Selection of software

US EPA's ECOSAR software was selected, for a number of reasons:

1. It has been developed with the intention for use in a regulatory context and has regulatory endorsement.
2. It is cited in the EFSA aquatic guidance document (EFSA 2013).
3. It contains models relevant to a broad range of chemistries including pesticides.
4. It is included in the OECD QSAR Toolbox.
5. It is freely available.
6. It is user-friendly.

Extraction of data

1. Pesticide metabolite data were extracted from the Pesticide Properties Database (PPDB 2014) = 675 metabolites.
2. Data filtered for metabolites with available experimental fish acute LC_{50} data = 350 metabolites.
3. Data filtered for metabolites with chemical identifiers recognised by ECOSAR = 189 metabolites.
4. Removed 3 inorganic compounds and one containing a metal atom = 185 metabolites.

Refinement of data

Metabolites were excluded where:

a. Experimental or predicted logKow values were outside of applicability domain of the QSAR*.
b. Experimental or predicted LC_{50} values exceeded the limits of solubility* (*allowing for 25% uncertainty in the logKow/solubility measurements).
c. "non-standard" endpoints reported (e.g. 24 hour LC_{50}).

= Final dataset of 150 metabolites.

Generation of predicted LC_{50} values

Metabolites were excluded where:

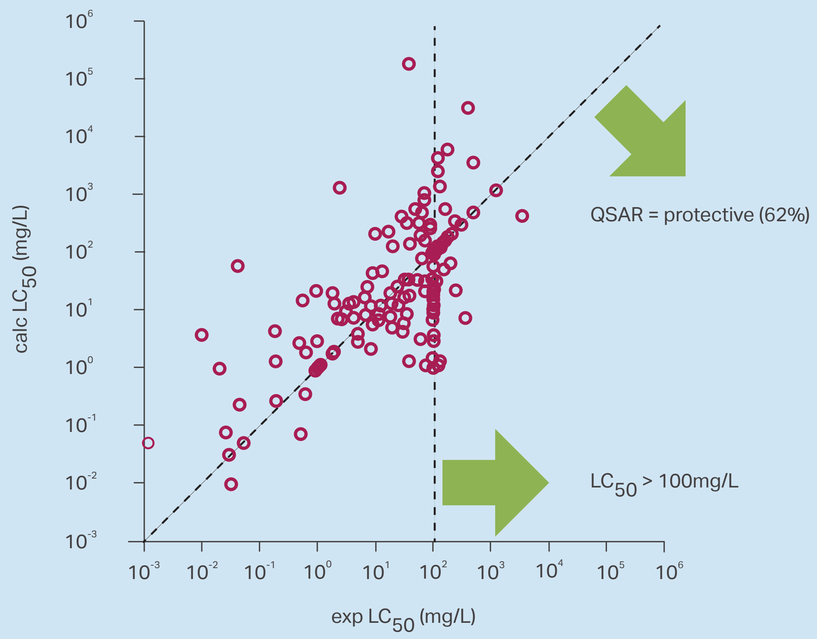
1. QSAR calculations performed for all 150 metabolites by inputting CAS number and/or SMILES code into ECOSAR.
   In many instances ECOSAR generated multiple predicted LC_{50} value based on different chemical classes ECOSAR also provided a baseline toxicity (narcosis) value for each chemical.
2. Predicted and experimental fish 96 hour LC_{50} values were entered into a spreadsheet.

Data analysis

Most conservative LC_{50} value generated for each metabolite selected for comparison to experimental LC_{50} (Figure 1).
60 metabolites had undefined experimental LC_{50} values (i.e. LC_{50} is > x mg/L). In these cases the predicted LC_{50} was normalised to this level if the prediction was above the experimental value.
The analysis was further refined based on quality scores ranging from 1-5 (with 5 representing the highest quality - "verified data used for regulatory purposes") within the database for the experimental data, and reasonable assumptions on experimental variability (Figures 2 and 3). Experimental and predicted 96-h fish LC_{50} data were compared graphically. The strength of the correlations was determined by Spearman's correlation coefficient. Predicted values which were greater than five times the experimental value, for data assigned the highest 'quality score' of five, were investigated further as potential outliers.

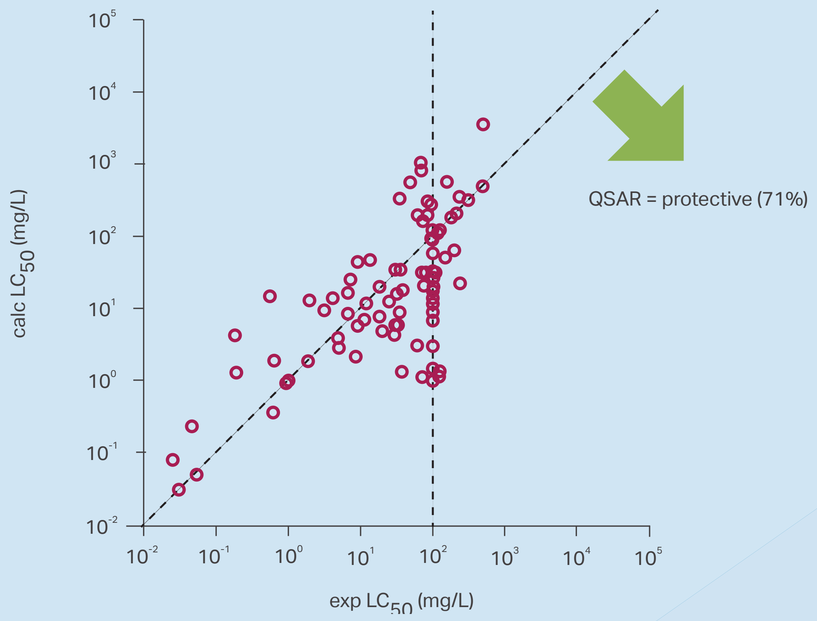
Results

Figure 1: All data



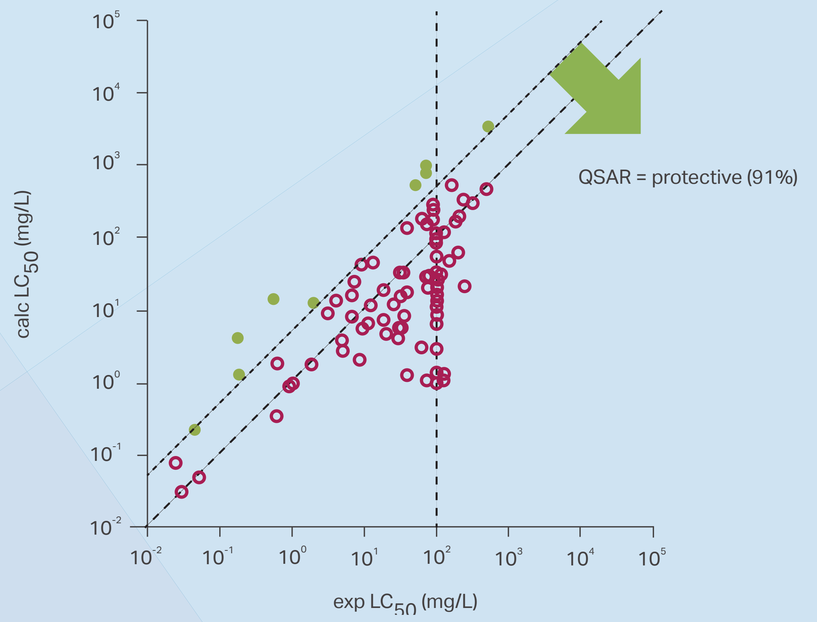
The line of unity shown on the graph demonstrates where predicted and experimental LC_{50} values are equal. 62% (93/150) of the predicted values fall on or below this line i.e. the predicted values are equal to or more conservative than those generated experimentally. Eight of the predictions which yielded higher LC_{50} values than experimental values were for non-toxic compounds (i.e. LC_{50} values > 100 mg/L), which would not be of regulatory concern. The correlation of experimental to predicted LC_{50} was calculated using Spearman correlation coefficient ($r = 0.63$, $p < 0.0001$; line not shown).

Figure 2: Assessment of highest quality studies



When only the metabolites where the experimental LC_{50} value has been assigned a quality score of 10 within the PPDB (i.e. regulatory data) are considered, the QSAR was protective in 71% (74/104) of cases; the correlation remained unchanged ($r = 0.62$, $p < 0.0001$, Spearman correlation).

Figure 3: Consideration of variability in experimental data



Applying a tolerance factor of 5 to account for potential experimental variability increases the proportion of protective predictions to 91% (95/104). Green data points represent potential outliers.

For the 10 outliers other relevant data e.g. from regulatory documents on the parent molecules was examined. For all the outliers except diclofop-phenol there is a plausible explanation for why the ECOSAR prediction is not a good estimate (i.e. > five times) of the experimental data.

Conclusions

- This is the first practical demonstration of the potential for QSARs to be reliably used in the prediction of the acute fish toxicity of pesticide metabolites.
- The employed QSAR models provide a conservative estimate of toxicity in the majority of cases. After taking into account data quality and potential variability of the experimental data and excluding the explained outliers, ECOSAR predicts the fish acute LC_{50} to be lower than or within a factor of five of the experimentally derived LC_{50} value in 99% of cases (93/94).
- Diverse chemistries and (parental) modes-of-action were included in the comparison. All available pesticide metabolites were included irrespective of whether the toxophore remained in the structure. Therefore, the strong correlation appears to hold with and without likely specific modes-of-action.
- This analysis supports the EFSA guidance for the use of QSARs to reliably predict the fish acute toxicity of pesticide metabolites. Thus, this method offers a non-animal testing pathway to generate data for environmental assessment of metabolites of concern (i.e. those identified as potentially relevant in environmental fate studies).
- We will disseminate the analysis and engage in a dialogue with regulators. This will inform the further refinements required before there can be widespread implementation.
- **Such implementation has potential to substantially reduce the number of animals used in the risk assessment of pesticide metabolites.**

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References & Acknowledgements:

- EFSA 2013. Guidance on tiered risk assessment for plant protection products for aquatic organisms in edge-of-field surface waters. EFSA Journal;11(7):3290.
- PPDB 2014. The Pesticide Properties DataBase (PPDB) developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, funded by UK national sources and through EU-funded projects, 2006-2014.