Investigation of the Applicability Domain of QSARs to Predict the Ecotoxicity of Pharmaceuticals

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Introduction

Improved methods of analysis have revealed low levels of active pharmaceutical ingredients (APIs) in surface and ground waters and in drinking water in the UK , leading to concern over potential adverse effects. APIs are not designed to produce acute toxicity, and levels are sufficiently low to make this unlikely, but their continual replenishment in environmental compartments raises particular concern about potential chronic effects.

There is a paucity of publicly available acute and chronic ecotoxicity data for APIs. Accessible data show wide variation in experimental protocols and results. Therefore, *in silico* methods to predict toxicity present an opportunity to gain further insight into potential effects. Tools such as ECOSAR have been used to predict ecotoxicity of APIs. However, ECOSAR was developed using simple industrial chemicals. This raises the question as to whether or not APIs fall within the applicability domain of the models. Further studies into potential mechanisms by which APIs may elicit ecotoxic effects are also of interest to aid understanding of the interaction of APIs with non-target environmental organisms.

Aims

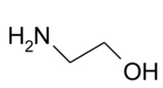
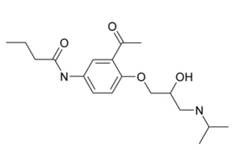
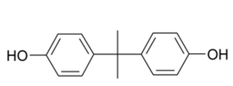
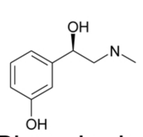
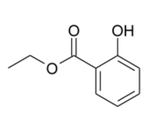
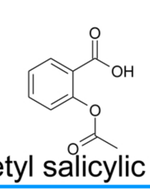
The aims of this study were:

- To use ECOSAR to categorise APIs into recognised chemical classes and assess the likelihood that the chemicals used to develop the ECOSAR models for those classes were representative of the APIs, hence to determine the reliability of the predictions
- To evaluate the publicly available ecotoxicity data for APIs and compare the experimental data with the predicted values.
- To investigate potential mechanisms by which APIs could elicit ecotoxic effects.

Methods

- A dataset of 364 common, well-characterised APIs was obtained[1,4,5,8]
- ECOSAR was used to categorise the compounds into recognised chemical classes and predict their ecotoxicity values. Based on chemical structure, a judgement was made as to whether or not the chemicals used to develop the ECOSAR model for that class was representative of the APIs assigned to that class. This allowed for an estimate of confidence in the predictions. Table 1 demonstrates this process for three representative compounds.
- Predicted and experimental values for fish, daphnid and algal toxicity (where available) were compared.
- To identify potential mechanisms of toxicity the dataset was analysed using the Verhaar rules as implemented in ToxTree[7] and an in-house program which uses SMARTS strings to identify those which may act via electrophilic mechanisms[3].

Table 1. Example compounds from ECOSAR classes and APIs allocated to those classes

ECOSAR class	Example from ECOSAR model	Example API allocated to class	Confidence in prediction
Aliphatic Amines	 Ethanolamine	 Acebutolol	Low
Phenols	 Bisphenol A	 Phenylephrine	Moderate
Esters	 Ethyl salicylate	 Acetyl salicylic acid	High

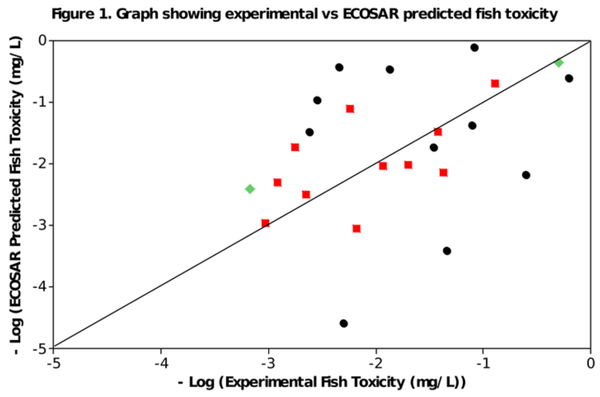
Results and Discussion

- Table 2 shows the majority of APIs were assigned to the aliphatic amine category, followed by neutral organic, ester and phenol categories. Based on structure, few APIs are likely to fall within the applicability domain for the respective models.
- For categories such as imides and hydrazines more APIs were likely to fall within the domain of the model, hence the predictions were more reliable.

Table 2. Classes to which APIs were allocated and no. times these were high / moderate confidence

ECOSAR class(es)[1]	No. APIs in group	High / mod confidence	ECOSAR class(es)[1]	No. APIs in group	High / mod confidence
Acryl amides	12	*	Imides	21	18
Acrylates	12	1	Neutral Organics	61	9
Aldehydes	1	0	Phenols	39	6
Aliphatic Amines	182	5	Propargyl Alc-hindered	1	0
Allylic/Vinyl Nitriles	2	0	Propargyl Amines	1	1
Anilines (2-amino)	6	0	Quinone/Hydroquinone	2	2
Aromatic Amines	19	1	Salicylic acid	2	*
Azides	1	1	Surfactants-cationic	1	*
Aziridines	1	1	Thiazolidinones	2	2
Benzyl Alcohols	21	1	Thiols(mercaptans)	1	1
Benzyl Amines	5	0	Thiophenes	6	4
Benzyl Halides	3	1	Triazines	1	0
Epoxides	1	1	Ureas (substituted)	3	*
Esters	46	11	Vinyl/Allyl Alcohols	15	2
Haloacetamides	1	0	Vinyl/Allyl Ethers	8	1
Hydrazines	9	6	Vinyl/Allyl Ketones	18	4
Imidazoles	24	5	* = no SAR for fish (96h), daphnid (48h) or algae (96h)		

Figure 1. Graph showing experimental vs ECOSAR predicted fish toxicity



low • moderate ♦ or high ▪ probability of being in domain

- Predictions for fish toxicity are more reliable for those compounds deemed to be within the domain of the models. The same trend was seen for both daphnid and algal toxicity (data not shown).
- Up to 10 fold variation was found in the experimental data, creating additional problems in building predictive models.
- Table 3 shows the results of the analysis into potential mechanisms of toxicity for the APIs in this study.

Table 3. Potential mechanisms via which APIs may elicit ecotoxicological efffects

% compounds allocated to groups in Verhaar Scheme		% compounds allocated to electrophilic mechanisms using SMARTS strings developed in–house[3]				
unspecific reactivity	cannot be classified	(pre) / Michael acceptors	acylating agents	(pro) / Schiff Base formers	(pro) / SN2	SNAR
38%	62%	14%	34%	17%	16%	1%

Conclusions

- Model users must ensure that training set compounds are representative of their query compounds as reliable predictions are only possible when compounds fall within the applicability domain of the models.
- APIs have the potential to elicit ecotoxicological effects via a range of mechanisms.
- Few APIs are categorised into appropriate classes by ECOSAR (ver 0.99g).

References

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