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Pharmaceuticals in the environment: Good practice in predicting acute ecotoxicological effects

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ABSTRACT

Improvements in analytical techniques have led to an increased awareness of the presence of pharmaceuticals in the environment. Concern is now raised as to the potential adverse effects these compounds may have on non-target organisms, particularly under conditions of chronic exposure. There is a paucity of experimental ecotoxicity data available for pharmaceuticals, hence the use of *in silico* tools to predict toxicity is a pragmatic option. Previous studies have used the ECOSAR program to predict environmental toxicity of pharmaceuticals, however, these models were developed using industrial chemicals and the applicability of the models to predict effects of pharmaceuticals should be carefully considered. In this study ECOSAR was used to assign 364 diverse pharmaceuticals to recognised chemical classes and hence predict their aquatic toxicity. Confidence in the predictions was assessed in terms of whether the assigned class was realistically representative of the pharmaceutical in question. The correlation between experimentally determined toxicity values (where these were available) and those predicted by ECOSAR was investigated in terms of confidence in the prediction. ECOSAR was shown to make reasonable predictions for certain pharmaceuticals considered to be within the applicability domain of the models, but predictions were less reliable for compounds judged to fall outwith the domain of the models. This study is not critical of ECOSAR or the class based approach to predicting toxicity, but demonstrates the importance of using expert judgement to ascertain whether or not use of a particular model is appropriate when the specific chemistry of a query compound is considered.

1. Introduction

An area of growing concern for both the public and scientists is the presence of pharmaceuticals in the environment and the potential adverse effects these may have. Although acute toxic effects are unlikely, continual exposure to low doses of pharmaceuticals may produce subtle, long-term effects on aquatic species. Of the existing environmental pollutants, studies targeted to determine the effects of pharmaceuticals have been relatively scarce (Daughton and Ternes, 1999). There is a need, therefore, to obtain a more detailed understanding of the adverse environmental effects of pharmaceuticals through either direct measurement, or accurate prediction, of their toxicological effects on non-target organisms.

Approximately 3000 pharmaceutical products are available for human use in the United Kingdom. The chemical properties of pharmaceuticals, engineered to resist rapid metabolism in the body to ensure adequate pharmacological effect, may also be responsible for their environmental persistence (Richman and Castensson, 2008). This is a topical issue due to the increasing awareness of the extent to which pharmaceuticals are present not only in surface and ground waters, but also in drinking water. Of the 61 drugs listed in Richman and Castensson (2008) as being present in environmental compartments, 15% were reported to have been detected in drinking water. Improvements in analytical techniques have lowered the limits of detection and quantification of pharmaceuticals, enabling accurate measurement of their concentration in different environmental compartments.

The scope of the problem presented by the presence of pharmaceuticals in the environment is demonstrated in the report by Ayscough et al. (2000), who provide a compilation of data including concentrations of pharmaceuticals detected in sewage effluent, surface and groundwaters. The authors include some environmental fate and toxicity information, however, consistent and reliable data are lacking in this area. Sanderson et al. (2004b) report that measured ecotoxicological data are available for <1% of pharmaceuticals. Data that do exist show wide variation between laboratories in experimental conditions, in results for given compounds and in species sensitivity (ECOTOX, 2008). Consequently, *in silico* models, specifically (quantitative) structure activity relationships ((Q)SARs), for predicting toxicity are becoming an increasingly attractive option, to fill knowledge gaps and to

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prioritise subsequent investigation of compounds deemed to be of greater concern, via incorporation into integrated testing strategies (Grindon et al., 2006). So far, models have been employed, by necessity, to provide some level of information (Boxall et al., 2000; Sanderson et al., 2003, 2004a,b; Jones et al., 2002; Ashton et al., 2004). Investigation into the appropriateness of these models for predicting effects of pharmaceuticals is important as is the continual development and refinement of these models as new data become available.

(Q)SARs have been used successfully for many years to model biological activity and toxicity of chemicals (Cronin, 2004). The ECOSAR program, freely downloadable from the US EPA website (ECOSAR, 2008) is a useful package for predicting the environmental effects of chemicals. This package allocates input compounds into one or more chemical classes. It then uses a hydrophobicity based SAR appropriate for the class(es) to make predictions for ecotoxicity. The logarithm of the octanol:water partition coefficient is used as the input parameter (which is calculated automatically should a measured value not be available). The SARs represent the correlation between chemicals' physicochemical properties and their aquatic toxicity. More than 150 SARs, covering over 50 chemical classes are incorporated in the models developed (Meylan and Howard, 1998). The assumption is made that the aquatic toxicity of a query compound can be predicted from known values for 'similar' compounds in the same class (ECOSAR, 2008). However, ECOSAR and other models to predict environmental effects have traditionally been developed using industrial chemicals rather than pharmaceuticals. Unlike industrial chemicals, pharmaceuticals by their nature are designed to be taken up into organisms, to avoid rapid metabolism and to elicit biological effects in mammals or bacteria. These features suggest that pharmaceuticals could produce effects on environmental systems if present in sufficient concentration. This raises the question of whether or not existing models, such as ECOSAR are suitable for predicting environmental effects of pharmaceuticals, as the chemicals used to create models should be representative of the compounds for which they are used to make predictions.

Confidence in the reliability of (Q)SARs is fundamental to their acceptance amongst end users. In terms of model development, the Organisation for Economic Cooperation and Development (OECD) offers guidelines for the validation of (Q)SARs. These OECD principles state that a model should have: (1) a defined endpoint, (2) an unambiguous algorithm, (3) a defined domain of applicability, (4) appropriate measures of goodness-of-fit, robustness and predictivity and (5) a mechanistic interpretation (if possible) (OECD, 2004). When using an established model to predict activities for new compounds, it is the third principle i.e. the definition of the domain of applicability, that becomes most significant. The prediction will only be useful if the compound falls within the applicability domain of the model. Whereas the other four principles are under the control of the model developer, it is implicit in the third principle that the end user is responsible for ensuring their query compound is within the applicability domain of the model. Unfortunately, in some cases models are used inappropriately to make predictions for compounds that are outside of the model's applicability domain. This can result in either incorrect predictions being reported, or the original model being criticised when it is found to perform poorly for certain compounds. Hence an explicit statement of the model's applicability domain or investigation of the appropriateness of the model for an individual query is essential.

In order to investigate the applicability domain of ECOSAR for pharmaceuticals, the aims of this study were to determine the chemical classes into which ECOSAR would assign a series of pharmaceuticals and to estimate the probability that the SAR for that class was appropriate to predict the toxicity for that pharmaceutical. This decision was based on whether or not the compounds within the class, on which the model was developed, were likely to be representative of the chemistry of the pharmaceutical. This enabled a ranking to be assigned to the confidence in the prediction, on a scale of 1–3 (where 1 is higher confidence), with respect to the probability of the toxicity being predicted accurately using the SAR for the class to which it was assigned. Using experimental ecotoxicological values for pharmaceuticals, where these were available, a comparison was made as to the accuracy of predictions for those pharmaceuticals considered to fall either within or outwith the applicability domain of the ECOSAR models.

2. Methods

Fig. 1 provides a schematic representation of the methodology used in this study to make a prediction of toxicity using ECOSAR and determine how appropriate the prediction is, with particular emphasis on the applicability domain of the model.

2.1. Data

A dataset of 364 pharmaceuticals was compiled from the literature and is given in Table 1. These compounds were selected as they comprised a highly structurally diverse series of drugs representative of a wide range of therapeutic groups. The majority of compounds were obtained from Goodman and Gilman's The Pharmacological Basis of Therapeutics (Thummel and Shen, 2001) which provides data for well-characterised drugs in common clinical use. Additional compounds for which ecotoxicological information were available (NCCOS, 2008; Webb, 2004; Cunningham et al., 2006) were also included in the dataset. Structures for all compounds were obtained from Thummel and Shen (2001) or from the ChemIDplus advanced website (ChemIDplus, 2008).

2.2. Assignment of pharmaceuticals to ECOSAR classes

Simplified Molecular Input Line Entry System (SMILES) strings (Daylight, 2008) were obtained for each compound by either manual generation or downloaded from the ChemIDplus advanced website (ChemIDplus, 2008). Each SMILES string was entered into the US EPA EPISUITE package KOWWIN (ver 1.66; EPISUITE, 2008) to obtain the logarithm of the octanol-water partition coefficient (log Kow). Experimental values were obtained wherever possible, otherwise the calculated log Kow values were recorded. (SMILES strings for all compounds, along with experimental or calculated log Kow values are available as Supplementary information). The SMILES strings and the log Kow values obtained from KOWWIN were entered into the EPISUITE ECOSAR program (ver 0.99g; ECOSAR, 2008) to predict aquatic toxicity. The class(es) into which ECOSAR assigned the pharmaceuticals was recorded for each compound (as shown in Table 1). In each case the class associated with the highest potency (i.e. the lowest concentration predicted to cause the toxic effect) is indicated by "b".

2.3. Ranking of the confidence in the ECOSAR predictions

The likelihood of the pharmaceutical falling within the applicability domain of the class was ranked on a scale of 1–3. If the compound was considered as likely to fall within the domain of the model, hence there was confidence that the SAR for that class could reasonably predict the toxicity of the compound then the confidence ranking was 1. If it was unlikely that the compound fell within the domain of the model and hence there was less confidence in the prediction for the compound, then the confidence ranking was 3. A ranking of 2 was used for compounds which could be within the domain of the model, but for which other factors may influence the toxicity, as outlined below and in Fig. 1.

The rationale for ranking a compound as 1, 2 or 3 was based on expert judgement taking account of several key factors, as indicated in Fig. 1. The number of classes into which ECOSAR allocated an individual pharmaceutical was one factor. Allocation to a large number of different classes is indicative of a relatively large molecule containing several functional groups. The presence of these different functional groups may moderate the toxicity of the molecule, hence it is unlikely that a single SAR based on one of the functional groups would accurately predict the toxicity of the molecule overall. Consequently compounds which ECOSAR allocated into four, five or six different classes were all ranked as 3, due to there being little confidence in the correct assignment of class. Similarly, compounds which ECOSAR allocated into three different classes were also generally ranked as 3 (in the case of five pharmaceuticals an exception was made to this rule for reasons explained in Section 3 below). Where a compound was allocated into one or two ECOSAR classes the likelihood of the chemistry of the classes being representative of the chemistry of the pharmaceutical was carefully considered. If similar compounds were present in the training set for ECOSAR the compound was ranked as 1. If the compound was similar to those in the training set, but contained an additional functional group that may have moderated the activity, a ranking of 2 was used. If the molecule was dissimilar to those in the training set (in terms of size, log Kow and/or presence of additional