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Improved *in silico* prediction of carcinogenic potency (TD50) and the risk specific dose (RSD) adjusted Threshold of Toxicological Concern (TTC) for genotoxic chemicals and pharmaceutical impurities

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ABSTRACT

The Threshold of Toxicological Concern (TTC) is a level of exposure to a genotoxic impurity that is considered to represent a negligible risk to humans. The TTC was derived from the results of rodent carcinogenicity TD50 values that are a measure of carcinogenic potency. The TTC currently sets a default limit of 1.5 μg/day in food contact substances and pharmaceuticals for all genotoxic impurities without carcinogenicity data. Bercu et al. (2010) used the QSAR predicted TD50 to calculate a risk specific dose (RSD) which is a carcinogenic potency adjusted TTC for genotoxic impurities. This promising approach is currently limited by the software used, a combination of *MC4PC* (www.multicase.com) and a Lilly Inc. in-house software (*VISDOM*) that is not available to the public. In this report the TD50 and RSD were predicted using a commercially available software, *SciQSAR* (formally MDL-QSAR, www.scimatics.com) employing the same TD50 training data set and external validation test set that was used by Bercu et al. (2010). The results demonstrate the general applicability of QSAR predicted TD50 values to determine the RSDs for genotoxic impurities and the improved performance of SciQSAR for predicting TD50 values.

1. Introduction

The Threshold of Toxicological Concern (TTC) was originally developed by the FDA Center for Food Safety and Applied Nutrition (CFSAN) for regulating food contact substances as the "Threshold of Regulation" for regulating food contact substances (FDA, 1995, 2002). The TTC is now applied to the regulation of genotoxic impurities in pharmaceuticals (EMEA, 2006; CHMP, 2008; FDA-CDER, 2008). The (TTC) is a level of exposure to a genotoxic impurity that is considered to represent a negligible risk to humans. For genotoxic impurities with no carcinogenicity data, the TTC currently sets a default limit of 1.5 μg/day in food contact substances and pharmaceuticals for an average adult weighing 70 kg. Impurities or food contact substances can be classified as genotoxic by conventional laboratory screening such as the Ames test or by computer-based structure-activity software such as *Derek for Windows* (*DfW*, www.lhasaltd.com), *Leadscope Model Applier* (www.leadscope.com), *MC4PC* (or *Toxlite*, www.multicase.com) or *SciQSAR* (www.scimatics.com) (US FDA, 2008). The TTC limit is derived from the results of rodent carcinogenic potency (TD50) values that were used to estimate lifetime cancer risk of 1 in 100,000 (Cheeseman et al., 1999; Fiori and Meyerhoff, 2002; Kroes et al., 2004). The TTC value is assigned to all genotoxic compounds with no adjustment for carcinogenic potency related to their structure–activity relationship to known carcinogens. A quantitative structure–activity relationship (QSAR) modeling approach was recently used to predict the carcinogenic potency (TD50) of impurities using a combination of *MC4PC* and *VISDOM* (Bercu et al., 2010). The predicted TD50 value was then used to derive the risk specific dose (RSD) that was proposed as an alternative dose adjustment of the TTC limit for genotoxic impurities. *VISDOM* is an Eli Lilly and Company proprietary software that is not available to the public, and this is a serious shortcoming to the general application of this approach. This limitation was recognized by Bercu et al. (2010) and in their report it was stated that "From the training data provided, predictive models can be developed using other well-established commercial or in-house software".

In this report the TD50 value was predicted using a single commercially available *SciQSAR* (formerly *MDL-QSAR*) software employing the same TD50 training data set and external validation test set that was used by Bercu et al. (2010) and distributed as supplemental data (doi:10.1016/j.yrtph.2010.03.010). This database was derived from the Berkeley Carcinogenic Potency Database (http://potency.berkeley.edu/). The results of this study

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demonstrate the improved performance of *SciQSAR* for predicting TD50 values, and eliminate the use of a proprietary software platform. The predicted TD50 value was then used to determine the risk specific dose (RSD) that is a carcinogenic activity based adjustment of the TTC classification of genotoxic impurities.

2. Materials and methods

2.1. SciQSAR software

The *SciQSAR* (*MDL-QSAR* version 2.3) software program used in this report was obtained from SciMatics Inc. (www.scimatics.com). This is one of several software systems employed by the US FDA Center for Drug Evaluation and Research (CDER) and CFSAN for regulatory and scientific decision support applications for a wide range of critical toxicological endpoints including mutagenicity, genotoxicity, and carcinogenicity. *SciQSAR* software contains an integrated set of tools for similarity searching, compound clustering, and modeling molecular structure-related parameters that includes 240 electrotopological E-state, molecular shape and connectivity, and other descriptors**.** These molecular descriptors can be statistically correlated with toxicological or biological endpoints. The goal of this research was to use *SciQSAR* software to develop predictive quantitative structure–activity relationship (QSAR) models for the TD50 endpoint and to compare the results to those published by Bercu et al. (2010) using the same rat and mouse training and external test data sets. The same *SciQSAR* (*MDL-QSAR*) software and similarity cluster analysis method used to estimate the TD50 values was also used to predict rodent carcinogenicity (Contrera et al., 2003) and the maximum recommended human daily dose (MRDD) (Contrera et al., 2004).

2.2. SciQSAR molecular descriptors and carcinogenicity database

E-state descriptors incorporate information related to atom types and electron accessibility that are influenced by all of the substructural features of a molecule (Kier and Hall, 1999a; Hall and Kier, 2001). The molecular meaning of molecular symbols for E-state descriptors used in this report are described in Kier and Hall (1999a,b) and Hall et al. (1995). For this study it was found that atom type E-state and E-state atom count descriptors generally produced the best models for TD50. E-state descriptors have been used to model biological endpoints (Contrera et al., 2003, 2005; Cash, 2001; Gini et al., 1999; Gough and Hall, 1999; Hall and Vaughn, 1997, Maw and Hall, 2000, Abou-Shaaban et al., 1996; Brown and Martin, 1996; Liu et al., 2001). E-state indices are useful molecular descriptors that can also be used as a chemical structure parameter for database searching (Kier and Hall, 2001). The array of electrotopological and other descriptors available in *SciQSAR* are more complex and diverse than traditional molecular fragment-based structural alerts (Ashby and Tennant, 1991).

Mouse and rat carcinogenicity training and external test database tables distributed by Bercu et al. (2010) as supplements 1 (mouse) and 2 (rat) were download (doi:10.1016/j.yrtph.2010.03.010) and used for this study. This database was derived from the Berkeley Carcinogenic Potency Database (http://potency.berkeley.edu/).

2.3. Structure similarity clustering and modeling

Similarity clustering identifies the primary molecular descriptors derived from the smallest set of reference training set compounds that are most structurally similar to a test compound and highly correlated to toxicological activity. These descriptors are not necessarily the only descriptors or structural attributes related to activity but represent an optimal set. This method is analogous to the least common denominator concept, and is used to optimize predictive performance and minimize model variability that can occur as a function of the size of a similarity cluster and the number descriptors present in a model.

The first step in the similarity clustering method is the identification of an optimal cluster of compounds from the control data set that is structurally most similar to a test compound. In this approach a unique QSAR model is generated for each test chemical from a cluster of structurally similar compounds derived from the training data set. The *SciQSAR* one touch similarity search tool employing *SciQSAR* E-State and E-State atom count descriptors and the Tanimoto similarity search algorithm was used to identify a cluster of training database compounds with at least 60% similarity to each of the validation test compounds. The subset of cluster compounds with the highest similarity index (usually the top 10–30 compounds) was then modeled for each test compound using the *SciQSAR* one-step genetic algorithm (GA) application which identified relevant descriptors. The GA method finds the "best" or "fittest" set of descriptors guided in part by optimization of the correlation coefficient (r^2) (Rogers and Hopfinger, 1994; Kubinyi, 1994a,b, Forest, 1993). The GA method sequentially generates sets of descriptors, and is modeled after the manner in which genetic information is passed from one generation to another. Selection of the best descriptors is accomplished through an algorithm which simulates mutation and genetic cross-over. Each set of descriptors (generation) is evaluated and its 'goodness of fit' determined by a set of criteria. Those descriptor sets with good fitness have the greatest effect on succeeding generations of descriptor sets. In this way, the algorithm makes use of the whole descriptor pool to select a set of descriptors with good regression statistics. An optimal cluster is the smallest set of cluster compounds that will yield good linear regression statistics with the least number of descriptors. When the Tanimoto similarity clustering fails to produce an adequate model the cosine coefficient similarity search method was used as an alternative that could yield good regression statistics. Compounds with poor regression statistics using either Tanimoto or cosine coefficient similarity clustering methods were not modeled and are classified as "not covered" (NC) compounds. These compounds are considered outside the domain of applicability (DA) of the database. The domain of applicability (DA) is partly a function of the molecular coverage of the test molecule relative to the molecules in the training data set. If a test molecule is not well-represented in the training data molecular library, the test molecule will be outside of the DA of the model and will have a poor regression statistics. In addition, some types of chemicals are unsuitable for current QSAR modeling due to their molecular composition, e.g., inorganic salts, large organic molecules (>1200 Da), polymers, fibers, organometallic chemicals, gases, and complex mixtures of chemicals.

The second step is the removal of inter-correlated descriptors (descriptors that are highly correlated to each other) and weak (statistical outlier) descriptors that are a major source of error and variability. The *SciQSAR* software identifies and highlights all inter-correlated descriptors in red and also identifies the relative significance and contribution of each descriptor in a regression equation. After inter-correlated and weak or outlier descriptors are deleted a new regression equation is generated, statistical analysis is performed and the results reanalyzed. The process of refining a model is repeated until a regression equation with good statistics and an optimal number of descriptors are attained. A characteristic aspect of similarity clustering is that a unique QSAR TD50 model is generated for each test compound rather than the usual single generalized (global) QSAR model that is applied to all test compounds.