Computer-Assisted Methods in Chemical Toxicity Prediction

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Abstract: *In Silico* predictive ADME/Tox screening of compounds is one of the hottest areas in drug discovery. To provide predictions of compound drug-like characteristics early in modern drug-discovery decision making, computational technologies have been widely accepted to develop rapid high throughput *in silico* ADMET analysis. It is widely perceived that the early screening of chemical entities can significantly reduce the expensive costs associated with late stage failures of drugs due to poor ADME/Tox properties. Drug toxic effects are broadly defined to include toxicity, mutagenicity, carcinogenicity, teratogenicity, neurotoxicity and immunotoxicity. Toxicity prediction techniques and structure–activity relationships relies on the accurate estimation and representation of physico-chemical and toxicological properties. This review highlights some of the freely and commercially available softwares for toxicity predictions. The information content can be utilized as a guide for the scientists involved in the drug discovery arena.

Keywords: Toxicity prediction, *In silico*, TOPKAT, LAZAR, DEREK, MULTICASE, HazardExpert, OncoLogic.

1. INTRODUCTION

In today's *in silico* age, information technology (IT) functions are essential in pharmaceutical industry, which is under extreme pressure to enhance productivity and cut costs associated with discovery and development of drugs. Toxicoinformatics, an *in silico* approach, emerged as an important area in toxicology field, and involves prediction of toxicity of chemical molecules in the living systems [1]. *In silico* drug discovery program has achieved a significant development for predicting new chemical entities (NCEs), by reducing the number of experimental trials required for pharmaceutical screening and selection. The present scenario is such that NCEs are abundant, but they need to be scrutinized critically, by unraveling various attributes like absorption, distribution, metabolism, elimination and toxicity (ADME/T), before forwarding them for Food and Drug Administration (FDA) approval and then to market.

The discovery of novel drug molecule consists of seven basic steps: disease selection, target selection, lead molecule identification, lead optimization, preclinical trial testing, clinical trial testing and pharmacogenomic optimization strategies. Out of all these steps, clinical trials is the most expensive stage [2]. Over 50% of drug like molecule failures are due to poor ADME/T profiles, and consequently, 20% of the total R&D costs per drug are spent to cross this barrier [1, 3]. Only 1 in 10,000 NCEs make it to the market. Even after such rigorous filtration with a high attrition rate, the toxicity profile of many molecules is not detected and consequently drugs need to be withdrawn from the market, presented in Table **1** [4-6]. Such drug failures lead to colossal loss not only in terms of time and money, but also patient health and security. Two main approaches for toxicity prediction include *in vivo* and *in vitro* analysis [7,8]. Both these approaches entail the synthesis of compounds before testing, which is not practical when dealing with large combinatorial library of molecules. Toxicoinformatics approach provides alternative tools by dint of which toxicity can be predicted, without veritably synthesizing the molecule. Admittedly, *in silico* approach helps us to surmount the immense public pressure to minimize the use of animals in toxicity testing, thereby supporting "Prevention of cruelty to animals act". It also helps in implementing the three R's theory of refine, reduce or replace animals in laboratory experiments propagated by the animal ethics committee [9]. However, animal testing is many times found to be slower than their non-animal equivalents and at times also is unreliable for toxicity analysis. In addition to providing guidance to synthetic chemists, the ultimate goal of toxicoinformatics is to significantly reduce animal testing with rapid *in silico* approaches for hazard (toxicity) characterisation and risk assessment [10,11]. Recent trend is an integrated *in silico* with *in vitro* and *in vivo* approaches to early ADME/T screening, for making effective decisions on NCEs selection, which will help support and accelerate drug discovery projects.

An overview of literature/scientific survey showed that different international groups/regulatory agencies are involved in toxicity prediction, such as European REACH program [12,13], environmental protection and regulatory agencies in USA, Danish, Canada, Japan etc. [14]. General and in depth information on toxicoinformatics exist in well known book "Predicting Chemical Toxicity and Fate" edited by Cronin and Livingstone, about *in silico* toxicity prediction tools and its applications in pharmaceutical, environmental and metabolic area [14]. The main theme of the present mini-review is to give brief update of different softwares, used for predicting *in silico* toxicity of chemicals and products, by giving its strengths and weakness, which can be utilized as guidance in the initial phase of the toxicological analysis.

IN SILICO APPROACHES FOR TOXICITY PREDICTION

There is a growing need for computational methods which can predict toxicological profiles and fate. Two basic approaches used in toxicoinformatics include (a) knowledge based systems (KBS) and (b) automated rule induction (ARI) systems. They differ fundamentally in the way they operate. KBS predict by reasoning on the basis of existing human knowledge whereas ARI systems make predictions by learning from and discovering patterns in existing data [14]. Some of the predictive toxicological endpoints computed using KBS and ARI systems include: rodent carcinogenicity, Ames mutagenicity, developmental toxicity potential, skin and eye irritation, acute oral toxicity LD50, acute inhalation toxicity LC50, acute toxicity LC50, acute toxicity EC50, maximum tolerated dose (MTD), chronic lowest observable adverse effect level (LOAEL), skin sensitisation. Predictive toxicologist use these end points, to check the toxicity profile of the NCEs or drug like molecules and prevent costly failures by identifying risky drugs, before they reach human testing. A general outlay showing the importance of these techniques for predicting structure-activity relationships (SARs) is presented in (Fig. **1**).

Automated Rule Induction (ARI) Systems Approach

Molecules from a training set of chemicals of known activity for a particular biological endpoint are fragmented into all possible atom pairs and other associations. Pattern recognition techniques are then used, together with other statistical analyses, to compare

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1389-5575/07 $50.00+.00

Table 1. Some of the Drugs Withdrawn from the Market due to Toxicity

Drug	Disease	Reason for withdrawal	Year of approval	Year of withdrawal
Azaribine	Psoriasis	Stroke	1975	1976
Nomifensine	Antidepressant	Hemolytic anemia	1984	1986
Encainide	Irregular heart beat	Fatal arrhythmia	1986	1991
Temafloxacin	Antibiotic	Kidney failure	1992	1992
Flosequinan	Congestive heart failure	Increased deaths	1992	1993
Mibefradil	Hypertension	Heart arrhythmia	1997	1998
Aestimizole	Anticholinergic, Antihistaminic	Heart arrhythmia	1988	1999
Rezulin	Antidiabetic	Hepatitis	1999	2000
Rofecoxib	Pain relief	Cardiac toxicity	1999	2004
Ximelgratan	Anticoagulant	Hepatotoxicity	2004	2006
Gatifloxacin	Respiratory tract infection	Diabetes	1999	2006

the frequency of occurrence of specific structural features in sets of active and inactive molecules. In this way, the most important features determining or modifying activity are identified. After it has been trained, the system can then be used to search for the presence of biophores and biophobes in novel molecules. Biophores are the substructure fragments that are determined to have a positive relationship with activity whereas biophobes are negatively related to activity. Some ARI utilize methods such as quantitative structure–toxicity relationship (QSTR). The basis for any QSTR is that the biological activity of a new or untested chemical can be inferred from the molecular structure, or properties, of similar compounds whose activities have already been assessed. Such QSTR models can be used to represent, explain, and most importantly predict property of interest i.e. toxicity. Attempts to quantify relationships between chemical structure and acute toxic potency have been part of the toxicological literature for more than 100 years. The toxicity of molecules is reflected in their structure. It was proposed that the biological activity, Φ, is a function, f, of constitution', C, of a molecule in a certain biological system.

$$\Phi = f(C)$$

QSTR models exist at the intersection of biology, chemistry, and statistics. In relating physico-chemical properties of structure to toxicity, the goal is to generalize from specific cases, to develop an understanding of what constitutes a 'class' of molecules that are active, what determines relative activity, and what distinguishes active from inactive molecules [14-16]. TOPKAT and LAZAR are amongst the best well known software's [17-19] following ARI approach.

Knowledge Based Systems (KBSs) Approach

KBS uses structural alerts in molecules, to develop rules devised by experts based on a database of previous information, for example, on different endpoints in toxicity. The rules describe toxicophores in molecules of known activity. A toxicophore [20] is a feature or group within a chemical structure that is thought to be responsible for the toxic properties. These structural features can then be identified in novel molecules drawn on the computer screen using commercially available chemical drawing packages, such as ISISDRAW and CHEMDRAW, or imported as standard mol file format. The efficiency of KBS crucially depends on the capability of experts to devise rules which depends on the availability of good quality toxicity data. KBS include softwares like DEREK, HazardExpert and OncoLogic [21-23] for predicting chemical toxicity and fate.

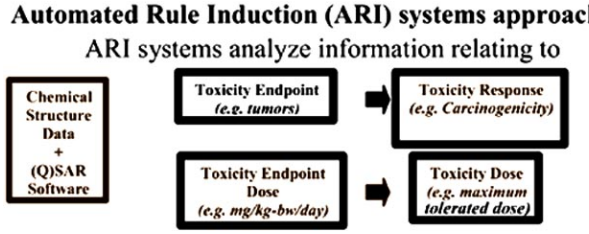


Fig. (1). The application of toxicoinformatics to analyze, model, and predict toxicological activity based on chemical structure-activity relationships (SARs).

Inverse Docking Approach

It is based on ligand (or drug)-protein inverse docking strategy such that a (query) molecule is attempted to dock to ligand binding pockets of proteins associated with potential toxicity and side effect. A molecule is considered toxic if it docks well into the protein site. The term "inverse" is used because the method is used for finding proteins that will fit with a specific ligand, rather than finding ligands that fit with a specific protein. INVDOCK algorithm follows this approach and its main applications are (i) Prediction of drug targets related to side effect and toxicity (drug safety evaluation). (ii) Identification of unknown and secondary therapeutic targets of drugs, drug leads, drug candidates, natural products, etc. (iii) Prediction of targets related to drug ADME (pharmacokinetics analysis). (iv) Identification of unknown receptors of a ligand (pathway analysis) [24, 25].

IN SILICO TOXICITY PREDICTION TOOLS

(I) TOPKAT- Toxicity Prediction by K(C)omputer Assisted Technology

TOPKAT was initially made by Health Designs and now developed and marketed by Accelrys [17]. TOPKAT accurately and rapidly assesses the toxicity of chemicals solely from their two dimensional (2D) molecular structure. It uses a range of robust, cross-validated QSTR models for assessing specific toxicological endpoints. It is characterized by verified databases, information-rich descriptors, highly predictive QSAR-based models and prediction-validation techniques which permits the user to determine the applicability of the model to the molecules being assessed. Each TOPKAT module consists of a specific database for predicting a specific toxicity endpoint.

A toxic response can be rationalized as a function of mainly two terms -the ability of the molecule to reach a site, the ability of the molecule to chemically interact with the biological system of

the site. TOPKAT uses descriptors to quantify the properties related to the transport of a chemical, e.g. molecular bulk, shape, symmetry, as well as descriptors that quantify the chemistry like the information-rich electro topological descriptors (E-state). This E-state descriptors quantify the electronic and topological attributes which is then used to quantify interaction at the site. TOPKAT prediction is generated through several sequential steps. First the chemical structure is entered in form of Simplified Molecular Input Line Entry System (SMILES) notations in SMILES entry form as shown in (Fig. **2**) and the relevant module (end point toxicity model) is selected. The test structure is then screened against sub-structural library for that module to check whether the test molecular structure is "covered" in the library, i.e. the query structure is already present in the database of submodel.

TOPKAT displays a message indicating its presence in database. After this toxicity prediction is formulated as shown, for example Ames mutagenicity prediction in (Fig. **3**). Continuous measures such as LD50, LC50, EC50, MTD and LOAEL are reported in weight/weight or weight/volume units whereas dichotomous measures, such as carcinogenicity, developmental toxicity and mutagenicity results are reported as a probability of 0-1. The user is also given literature references to the original sources of information. Any model may be applied to any query structure, and TOPKAT will provide a numerical answer. The prediction is reliable when the query is within the optimum prediction space (OPS) which is a unique multivariate descriptor space in which the model is applicable. If a query structure is outside OPS it may not necessitate that the TOPKAT assessed value is incorrect; in fact, the value may be extremely accurate or correct. TOPKAT has the capability of examining the contribution of any contiguous structural moiety i.e. a specific atom or group of atoms selected. This feature displays a colour-coded "toxicity map" of the query structure. Moiety analysis module also provides a quantitative estimate of toxic effect of the moiety. For example, DEREK mutagenicity model for bisfuranoids is well known and its toxicophore is shown in bold face (Fig. **4**-above) [26]. Using Ames mutagenicity model in TOPKAT we have performed moiety analysis of bisfuranoid mycotoxin substructure. The colour coded toxicity map of this substructure showed positive

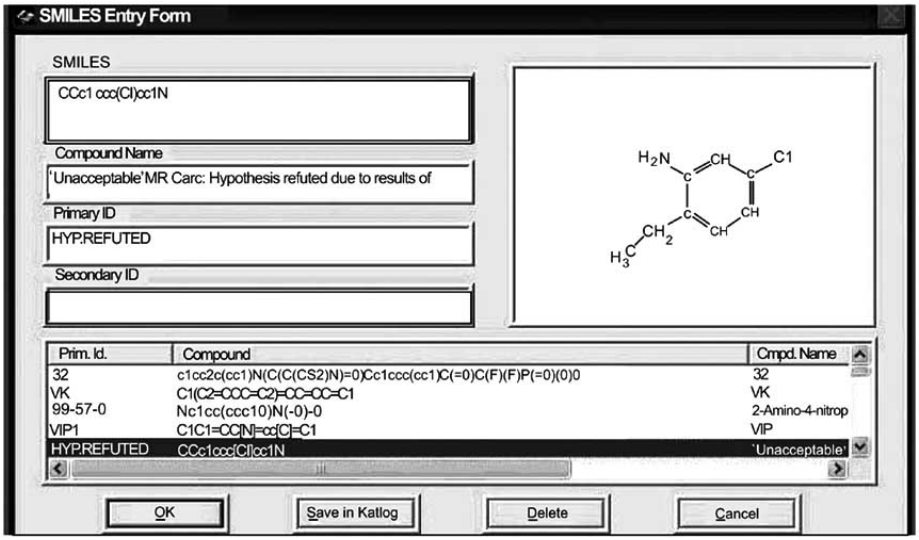


Fig. (2). SMILES entry form in TOPKAT showing structure.

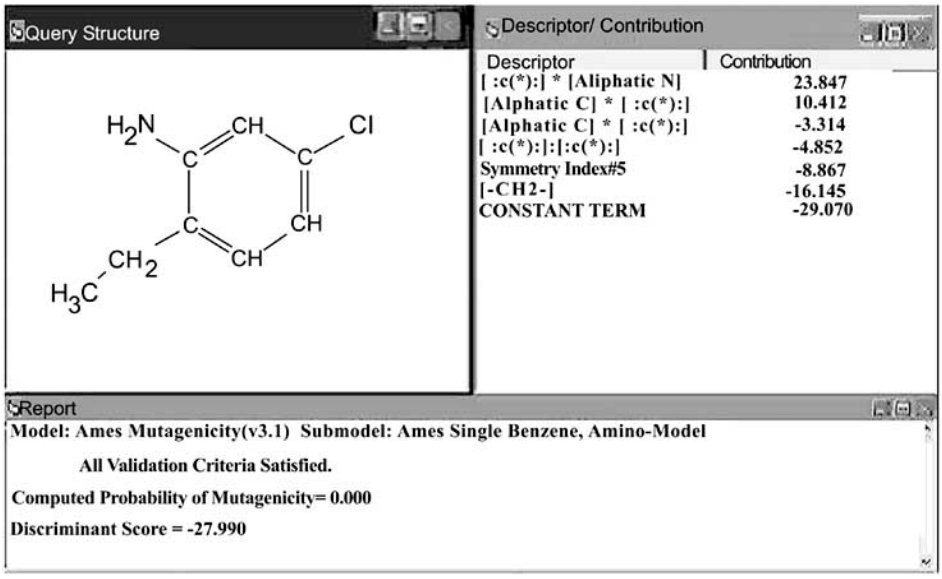


Fig. (3). Topkat interface after performing Ames mutagenicity model test.

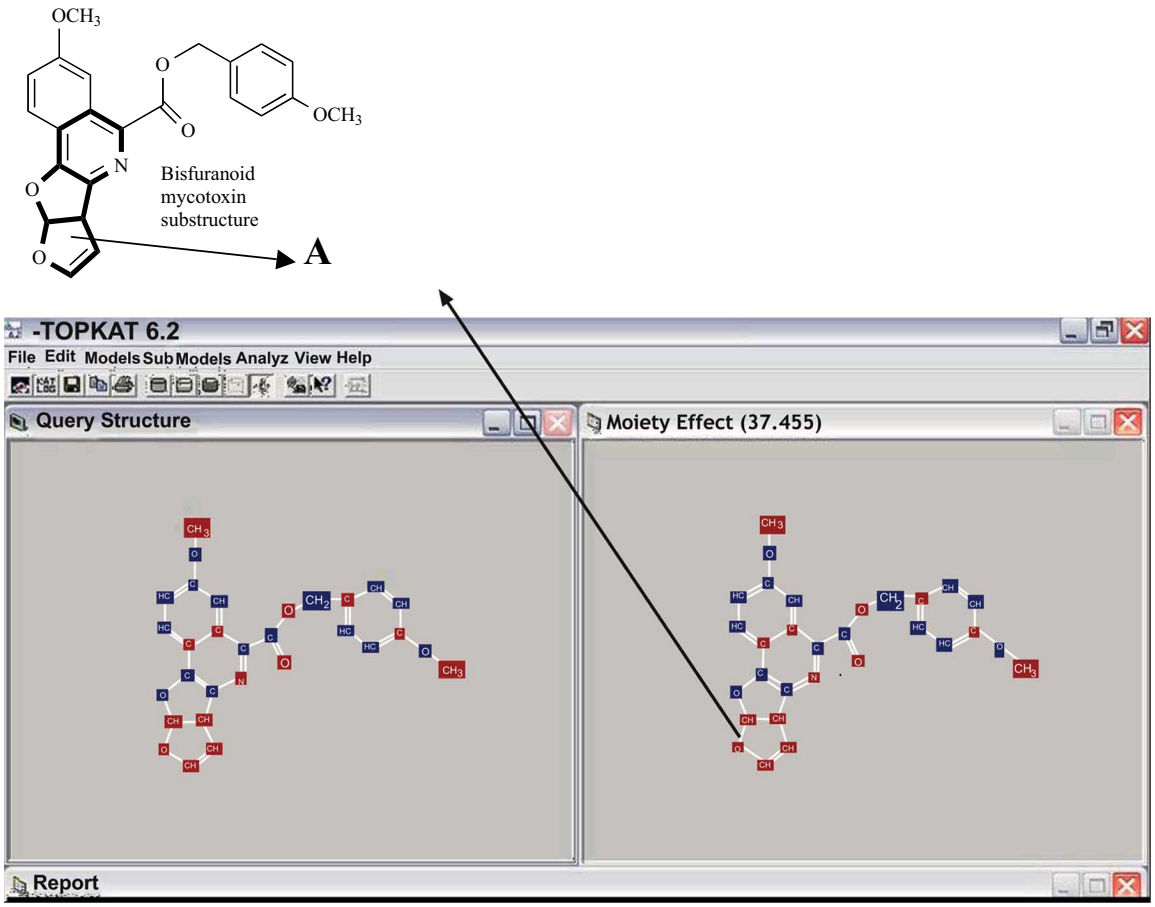


Fig. (4). Mutagenicity toxicophore shown in bold face for bisfuranoids (bisfuranoid mycotoxin substructure) in DEREK, (above) [26] and Ames mutagenicity toxicity map for bisfuranoid mycotoxin substructure in TOPKAT (below) [17].

contribution towards toxicity to be 37.45 and probability of being mutagenic 1 (i.e.100%) (Fig. **4**). Five membered ring A in this substructure was predicted to contribute towards mutagenicity in both DEREK and TOPKAT (Fig. **4**-above and below). There is difference in toxic substructure prediction among these two softwares. DEREK predicted toxicophore of this substructure to have three rings (two five membered ring and one six membered ring), shown in bold face. But, TOPKAT gave in addition to ring A, the carbonyl and two methyl groups of the substructure to be mutagenic (Fig. **4**-below). This analysis alert synthetic chemist for modification to be made in the ring A of bisfuranoid mycotoxin substructure, in order to eliminate its toxic effects.

In its current form TOPKAT does not allow users to implement their own models within the system. Moreover the databases used are intangible. The output interface comprises three areas showing query structure, descriptor contributions and report of the result.

TOPKAT provides a means of validating the toxicity assessment through similarity searching. The underlying assumption is that if the model performs an accurate assessment for the similar molecule from the database, then the model should also perform an accurate and valid assessment of the query structure. TOPKAT offers some advantages over other commercial softwares such as (i) It is highly user friendly and windows based software, (ii) It offers variety of toxicity prediction modules, (iii) It is highly time efficient, (iv) It is automated to choose the specific chemical class submodel, (v) After running a prediction, TOPKAT informs the user whether the prediction is within the OPS or not, reflecting the degree of confidence to be assigned, (vi) Use of batch mode operation when dealing with many molecules.

Present limitations in TOPKAT are (i) Chemical structure assessments (long-chain aliphatic, polymers and complex ring structures) are not well covered by certain TOPKAT modules. It is not possible to generate TOPKAT predictions for chemical structures comprising salts, inorganic or enol-keto forms, or for iodinated chemicals. (ii) The assumption that sub-structural features contribute independently to biological activity, is not always the case. (iii) The Fathead Minnow model has no sub-models for hetero aromatics and single benzenes with four or more substituents. (iv) Compounds of the following types: cyclopropenone, cyclobutendione, and cyclopententrione will not be assigned a sub-model for the following models: Ames mutagenicity, development toxicity potential, Rat oral LD50, Rat inhalation LC50, Rat maximum tolerated dose, Chronic LOAEL, Skin irritancy, Skin sensitization, Ocular irritancy, Aerobic biodegradability, and Fathead Minnow LC50. (v) The VLogP model does not support similarity searching. As the VLogP model has a great number of "descriptors" (or contributors) in its model, it is difficult to decide which descriptors to use for calculating similarity. (vi) The choice of organic elements, charges, hybridization states is limited so is the number of non-hydrogen atoms (105), rings (9), characters in SMILES (249), fragments (10) in a molecule. (vii) Bonds between separate fragments are not handled, e.g., C1.C1 will not be read as ethane, but as methane with an open ring connection (which will fail due to the missing ring closure).

(II) LAZAR- Lazy Structure-Activity Relationships

LAZAR (ARI system) is a novel tool in toxicoinformatics field and is available freely online. It is very useful for the prediction of toxic properties of chemical structures [19] and derives predictions for query structures from an inductive database which contains experimentally determined toxicity data. The predictive power of LAZAR mainly depends on the high quality data fed in its inductive database. Apart from the toxicity prediction, it provides the rationales (structural features and similar molecules) for the prediction and a reliable confidence index that indicates, if a query structure falls within the applicability domain of the training set. The input window of LAZAR is shown in (Fig. **5**).

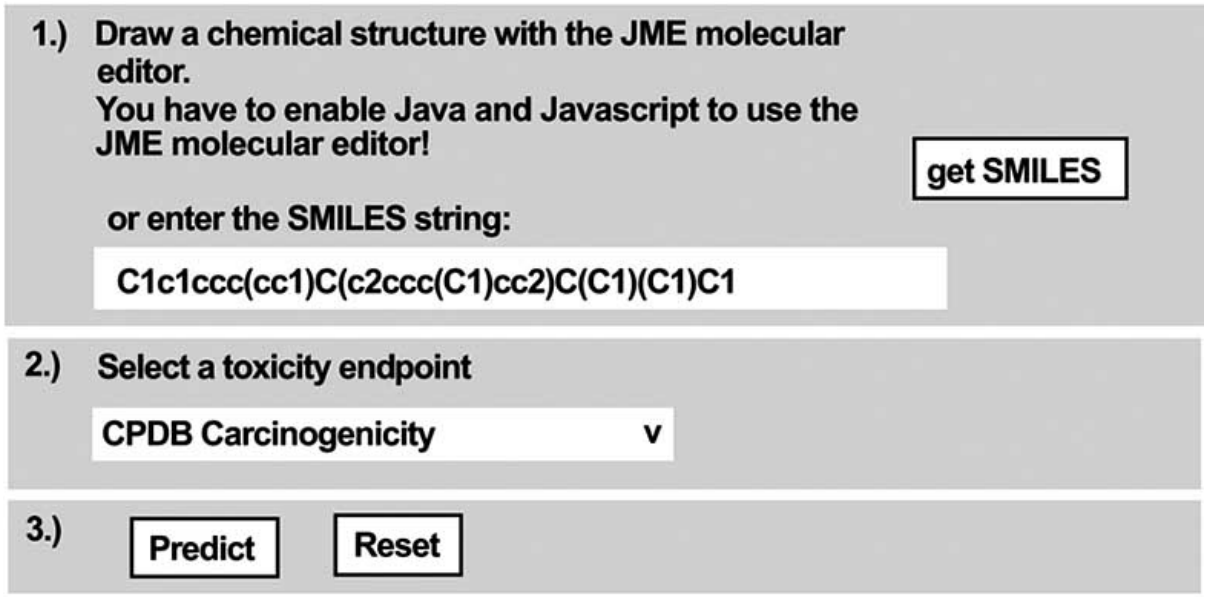


Fig. (5). Input window of LAZAR.

LAZAR derives its prediction specifically for a query structure using a modified k-nearest- neighbour (k-nn) algorithm. For this purpose it searches a database with chemical structures i.e training set and its experimental data which are similar to the query structure (neighbours) and calculates a prediction from the experimental measurements of the query structure. At present, LAZAR uses the language of linear fragments for the identification of toxic substructures of the query molecule. Linear fragments are defined as chains of heavy (non-hydrogen) atoms with connecting bonds, without branches or cycles. All linear fragments that are present in the query structure or in one of the training structures are determined exhaustively by molecular feature miner (MOLFEA) algorithm. MOLFEA mines for fragments in chemicals and this step does not consider its biological activities. Although linear fragments seem to be limited at a first glance (no explicit consideration of branches or cycles), they perform remarkably well on a variety of toxicity endpoints. A possible reason is that a lot of chemical information is implicitly contained in these fragments and the "chemical context" is considered by the k-nn based prediction algorithm.

The goal of the feature selection step is the identification of fragments that are relevant for the toxic activity under investigation. Significance of the results is determined using chi-square test and fragments below a predefined threshold are discarded from further calculations. Training set with a similarity above a predefined threshold are considered as neighbors to the query structure. Results are reported in the format as in (Fig. **6**). To classify a query structure one can seek confidence measure, conf, which indicates the expected class and the reliability of the prediction. Query structure is classified as active, if conf > 0 and as inactive, if conf < 0.

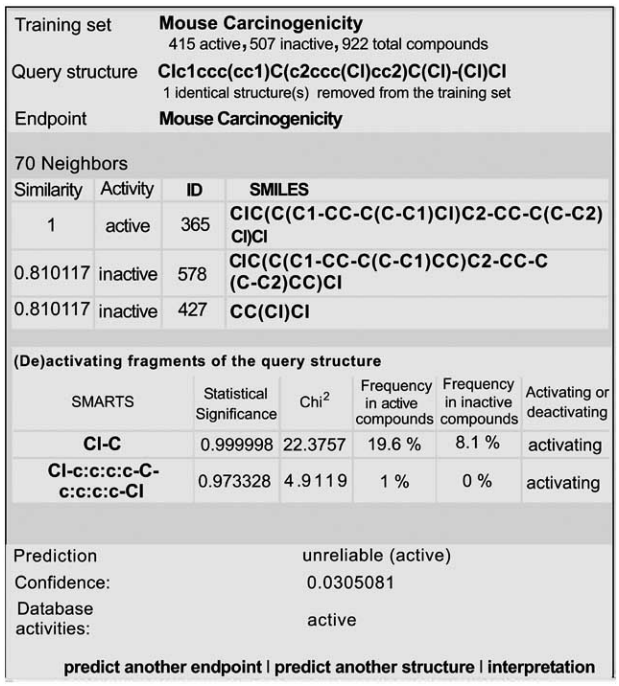
(III) DEREK for Windows- Deductive Estimation of Risk from Existing Knowledge

Derek for Windows, a knowledge-based expert software system which is marketed by the not-for-profit organisation Lhasa Limited [21], was originally devised at Schering Agrochemicals. It makes qualitative predictions about the activity of a query molecule. Derek for Windows has several rule-bases, containing descriptions of molecular substructures (structural alerts), which have been associated with toxic endpoints on the basis of existing knowledge.

The rules are generic in nature, i.e., they are based on sets of related chemicals rather than on specific chemicals. The rules cover a broad range of toxicological endpoints and the main strengths of Derek for Windows include prediction of mutagenicity and carcinogenicity. Reasoning rules are based on: If (Grounds) is (Threshold) then (Proposition) is (Force). *Grounds* may be any particular property. When the value of the property crosses a *threshold* then the *proposition* which represents the toxicological endpoint is *force* i.e. it becomes likely that the chemical is toxic. The reliability of these predictions is presented in the form of one of eight levels of likelihood. Reasoning models are mathematically based on the logic of argumentation.

The molecule query is input to the program by drawing or importing its two-dimensional topographical structure. Derek for Windows then searches the rule–base, highlighting the alert substructure located within the query structure, and a message indicating the nature of the toxicological hazard is then provided. Structural alerts are often supported by relevant literature references. Derek for Windows uses the MDL ISIS/Draw package as its molecular editor. It allows batch processing; using the MDL standard SDfile format. It also provides a graphical editor for users to add new rules.

The main strengths of the Derek for Windows software: (i) Development of the peer reviewed rules, (ii) The graphical interface is highly user-friendly, highlighting the toxicophore and displaying the relevant literature references, (iii) Graphical rule editor provides an easy way to add new rules but at the same time illicit modifica-



Training set **Mouse Carcinogenicity**
415 active, 507 inactive, 922 total compounds

Query structure **ClC1ccc(cc1)C(c2ccc(Cl)cc2)C(Cl)-(Cl)Cl**
1 identical structure(s) removed from the training set

Endpoint **Mouse Carcinogenicity**

70 Neighbors

Similarity	Activity	ID	SMILES
1	active	365	ClC(C(C1-CC-C(C-C1)Cl)C2-CC-C(C-C2)Cl)Cl
0.810117	inactive	578	ClC(C(C1-CC-C(C-C1)CC)C2-CC-C(C-C2)CC)Cl
0.810117	inactive	427	CC(Cl)Cl

(De)activating fragments of the query structure

SMARTS	Statistical Significance	Chi^2	Frequency in active compounds	Frequency in inactive compounds	Activating or deactivating
Cl-C	0.999998	22.3757	19.6 %	8.1 %	activating
Cl-c:c:c:c-C-c:c:c:c-Cl	0.973328	4.9119	1 %	0 %	activating

Prediction: unreliable (active)
Confidence: 0.0305081
Database activities: active

predict another endpoint | predict another structure | interpretation

Fig. (6). Output result format of LAZAR.

tion of the system is prevented by the use of license key, (iv) There is a batch processing feature, (v) There is considerable flexibility for adding data to the database, (vi) The user can access the information used to formulate the rule base, i.e., the database, references, and other supporting information (including relevant statements).

Its limitations are: (i) The lack of default display of C and H atoms, may be confusing to the non-chemist. [21,22,23], (ii) It does not provide elaborate information about the activating and detoxification effects of metabolism,. However, a link to sister product Meteor provides information about the possible metabolites of the query compound thus assisting Derek for Windows in detecting their toxic potential.

(IV) HazardExpert

HazardExpert is an ARI expert system [22]. The software has open architecture, in other words, the chemists, toxicologists, drug disposition experts or environmental managers can understand, expand, modify or optimize the data on which the toxicity estimation relies. Chemical structures can either be selected from a database or, if it is a new chemical, the user has to enter the structure into the database before a prediction can be made. The user is required to define the species, dose level, route and duration of exposure.

The query structure is searched for known toxicophores that are derived from literature in the field of QSAR or from the US EPA and Interagency Testing Committee (ITC) monographs. The substructures that exert both positive and negative modulator effects are stored in the 'Toxic Fragments Knowledge Base'. Knowledge maintenance module helps to add new structure and new toxic fragments to database and knowledge base respectively. Estimates for a number of toxicity endpoints are made once a toxicophore has been identified. The rules describe toxic segments and their effects on various biological systems. They are devised by integration of toxicological knowledge, expert judgment, QSAR models, and fuzzy logic (which simulates the effects of different exposure conditions). HazardExpert predicts the pKa and logP values of the molecule, and uses these for calculation of bioavailability. Approximately it contains 100 toxic rules and every rule is globally defined.

The resulting predictions are given in form of histogram with toxicity classifications. The toxicity is predicted on a 0-100-percentage scale in each toxicity class. Since this number is not a very precise value, the final result is given in probability categories which are classified as: highly probable toxic, probable toxic, possible toxic, uncertain toxic, not toxic. HazardExpert incorporates some reasonable estimates of physicochemical properties in its predictions and also provide estimates for bioavailability and bioaccumulation. It provides semi-quantitative estimates for toxicity. Besides these advantages there are few limitations which includes the inability to provide quantitative of metabolites, for novel datasets further 'validation' studies are required to increase the confidence in the predictions.

(V) MULTICASE- Multiple Computer Automated Structure Evaluation

The Computer Automated Structure Evaluation (CASE) technology refers to a range of different programs supplied by MULTICASE Inc. (Cleveland, OH, USA), known as ToxAlert, CASE, Multi-CASE, and CASETOX. MULTICASE is a hybrid of 2D QSAR and artificial expert structure based program [29]. Chemical structures are entered using either of the KLNor SMILES line notations, or from three types of structure files, Clark Still, MDL MOL file and SYBYL MOL files. SMILES code is to be preceded by "S" i.e. example Sc1ccccc1 for benzene and should not be longer than 180 characters. It allows the user to create or modify database. Size of database has nothing to do with number of chemicals to be tested. The best method to test a database is to test a set of chemicals that are not contained in the database and whose experimental results are available. If the database is good, it will give a high percentage of true predictions for both active and inactive chemicals. The structure of each molecule is divided up into all possible fragments, of two to ten heavy (non-hydrogen) atoms in length. Statistical methods are then used to classify the fragments as biophores or biophobes,:

The output from MULTICASE is in textual format displaying the physicochemical properties calculated for the query and the presence of active or inactive fragments in the query molecule [29-31]. For example, to predict carcinogenicity in rodents, databases for carcinogenicity assay need to be accessed. The module MCASE or CASETOX searches the structure of the chemical in the database for the presence of one or more biophores.

If a biophore is present, then MCASE predicts the chemical is active, otherwise inactive. The statistical measure of the predictive value of biophore distribution in the database is taken into account. The system also alerts the user to the presence of fragments in the query molecule that were not present in the training set. The quality of testing large and structurally diverse library of chemicals must depend upon the diversity of chemicals present in its database. The result is represented in CASE units in the range of 10-99; with value 10-19 indicating inactivity, 20-29 indicating marginal activity, 30-99 indicating increasing activity.

$$\text{CASE units} = \text{constant} + a(\text{Fragment 1}) + b(\text{Fragment 2}) + \ldots..$$

The confidence level in the biophore is given as probability. Other features of MULTICASE include the ability to batch process structures. It also has links to the META program for metabolism prediction.

Main strengths of this software is (i) Predictive models can be generated even for those molecules for which knowledge of mechanism of action is unknown, (ii) The predictions are modulated by a number of physicochemical properties, (iii) Batch processing of

molecules is very fast once the input files have been generated. Its limitations are (i) The system often fails to distinguish between molecules containing several small chains within one complex fragment from other molecules containing the same fragments distributed separately, (ii) The quality of the predictions made by the system is closely linked to the quality of the data used in the training set, (iii) Output from the software is often ambiguous and can lead to misinterpretation of the predictions, (iv) Training set has to be purchased separately.

(VI) Other ADME/T Prediction Tools

Softwares for predicting ADME/Tox related properties cover a wide range and among them QikProp [32,33] and VolSurf [34,35] is well known for high- throughput screening from medium to large compound databases. These tools are indispensable in lead discovery and development.

QikProp has the capability for predicting ADME/Tox properties such as - octanol/water and water/gas log Ps, log S, log BB, overall CNS activity, Caco-2 and MDCK cell permeabilities, human oral absorption, log K_{hsa} for human serum albumin binding, and log IC_{50} for HERG K+-channel blockage. It also has the ability to check the Lipinski Rule-of-Five and Jorgensen Rule-of-Three violations for a compound to be drug-like [32,33]. All these property prediction of a NCE decide its suitability for lead generation and its optimization.

VolSurf [34-36] predicts ADME properties using pre-calculated models, computes unique ADME descriptors which quantitatively characterize size, shape, polarity and hydrophobicity. It performs statistical analyses to generate predictive models of bioactivity or property of compounds including DNA fragments, peptides and proteins. The main advantage of VolSurf is the rapid predictions for use with virtual screening tools. Structure-property models obtained using Volsurf -ADME descriptors are significantly more predictive and also supplement QSAR with CoMFA's built-in 2D and 3D descriptors**.** These models do not require molecular alignment, translation of GRID or CoMFA fields into chemically intuitive descriptors and also the models are insensitive to conformational sampling. VolSurf was successfully validated in different systems, such as, membrane partitioning of oligopeptides, blood-brain barrier permeation, the anti-HIV activity of quinolones and oral availability.

Brief Overview and Application of Toxicoinformatics Softwares

General outlay of various well known toxicoinformatics softwares described above are summarised in Table **2**. A comparative analysis of these softwares, with its functioning capacity, would provide an idea of selecting the best available expert system, for different toxicology/medicinal chemistry projects (Table **3**).

These analysis would also help toxicologist and medicinal chemist to identify the relevant module of the expert systems, for the prediction of specific toxicity endpoints of the model, for their general or more specific case studies. Review of literature survey shown good amount of predictive toxicity models generated by using these softwares. An integrated testing strategies involving *in silico,* with *in vivo* and *in vitro* model analysis, in most of the above mentioned end points, is the latest trend, for early ADME/Tox

Table 2. Some of the Freely and Commercially available Toxicoinformatics software's for Toxicity Prediction

Software/ Supplier/Status	Toxicity Endpoints Predicted/ Associated Toxicity Databases	URL
LAZAR/ *In silico* toxicology/ (FREE)	Rodent carcinogenicity, {Hamster| Mouse|Rat} carcinogenicity, Salmonella mutagenicity, Fathead Minnow Toxicity (LC_{50}), FDA human liver toxicity/ CPDB and DSSTox US EPA	www.predictive-toxicology.org/lazar/
TOPKAT/ Accelrys Ltd./ (COMMERCIAL)	Carcinogenicity, Mutagenicity, Developmental toxicity, Rat LD_{50}, Rat chronic LOAEL, Skin sensitization, Skin irritancy, Aerobic biodegradability, Ocular Irritation, Fathead Minnow LC50, Daphnia magna EC50, VlogP/ US FDA, US NCI/NTP, US EPA, CDER, RTECS,GPMT, MITI, Draize and AQUIRE	www.accelrys.com/products/topka
DEREK for Windows/ LHASA Ltd./ (COMMERCIAL but not for profit)	Mutagenicity, Carcinogenicity, Skin sensitization, Irritancy, Lachrymation, Neurotoxicity, Thyroid toxicity, Teratogenicity, Respiratory sensitization, Acute toxicity and many other effects (over 40 endpoints in version 9) / Knowledge based system	www.lhasalimited.org
MCASE, CASE, CASETOX/ MultiCASE Inc./ (COMMERCIAL)	Carcinogenicity, Teratogenicity, Mutagenicity, Irritation, Maximum tolerated dose, Short-term genotoxicity, Biodegradation, Various mammalian acute and chronic toxicities and many other effects/ Knowledge based, US FDA	www.multicase.com
ToxScope/ Lead Scope Inc./ (COMMERCIAL)	Carcinogenicity, genetic toxicity, irritation, Hepatotoxicity: reproductive, subchronic liver and many other mammalian toxicological endpoints/FDA CDER Chronic/subchronic, Genetox and CFSAN Genetox	www.leadscope.com
Hazard Expert/ Compu Drug/ (COMMERCIAL)	Carcinogenicity, Teratogenicity, Oncogenicity, Mutagenicity, Membrane irritation, Neurotoxicity, immunotoxicity, bioavailability, bioaccumulation/ US EPA	www.compudrug.com
Tox Boxes/ Pharma Algorithms Inc./ (COMMERCIAL)	Acute toxicity, Genotixicity, Organ-specific health effects/ Ames database, Data from chronic, subchronic, acute and carcinogenicity studies from various species and routes of administration. LD50 data from six different animal systems:mouse and rat – intraperitoneal, intravenous, subcutaneous, oral administration/ Ames Test and Solubility Database.	www.ap-algorithms. com/
OncoLogic/® (FREE)	Cancer Expert System or OncoLogic ® Carcinogenicity/ US EPA, IARC, NCI/NTP, CPDB etc.	http://www.epa.gov/opptintr/newchems/pubs/sustainablefutures.htm

NCI: National cancer institute, CPDB: Carcinogenic potency database, DSSTox: Distributed structure-searchable toxicity database, NTP: National toxicology program, EPA: Environmental protection agency, CDER: Centre for drug evaluation and research, RTECS: Registry of toxic effects of chemical substances, GPMT: Guinea pig maximization test, MITI: Ministry of international trade and industry, IRC: International agency for research on cancer.

Table 3. Comparative Analysis of Different Toxicoinformatics Software's

ARI Based Expert Systems	KBS Based Expert Systems
TOPKAT ← MULTICASE/LEADSCOPE →	DEREK
QSAR based and collects molecular fragments and descriptors	Inspects molecules for known structural liabilities
Calculate chemical descriptor values	Identifies structural liabilities
Comparison of similar molecule from the database	Prepares summary report of findings
Using multivariate statistical analysis to predict the probability of being a member of toxicity class	Validated structural relationships with known toxic mechanisms
Structural liabilities identified	Provides references & predicted mechanisms
Non-validated structural relationships	
Provide relative dose and liability prediction	Chemically intuitive results
Easy to determine if molecule is well represented in training set via similarity search	Good initial filter for known liabilities: Lacks specificity
Can be biased to minimize false positives and/or false negatives	Only predicts presence of identified fragments
Challenging to systematically improve model: No linearity	Cannot discriminate within a structural sub-class
Difficult to Train General Model: Excellent predictiveness for single event; Problematic for multiple events	Retrospective in nature
Toxicity endpoint prediction is usually based on mechanism of action	Cannot extrapolate prediction to NCEs
Good For Specific Models	Good For General Models

screening in drug discovery, reviewing of it is beyond the scope this article. Various human and environmental health end points involving *in silico* (ARI and KBS) models are briefly reviewed alongwith the associated softwares.

(i) The prediction of eye and skin irritation have been reviewed by Patlewicz *et al.* (2003) [37]. Due to the lack of quality *in vivo* data, the QSAR predictive power is limited in this area. Both TOPKAT and MultiCASE contain models to discriminate between irritants and non-irritants. Also DEREK and HazardExpert is coded with a number of rules, mainly relating to strong acidic and basic molecular features which may be relevant for eye and skin irritation study [37]. (ii) The QSARs of acute toxicity end point have been reviewed by Lessigiarska *et al.* (2005) [38]. There are limited number of models in this area involving regulatory data. Some neural network studies have undergone in more complex non-linear model data. TOPKAT and MultiCASE contain acute toxicity models and DEREK also lists few rules for its prediction [38]. (iii) QSARs of chronic toxicity end point prediction are very limited. TOPKAT and MultiCASE has its models and DEREK have some rules related to organ toxicity [39, 40]. (iv) Variety of models exist in mutagenicity end point prediction which are more specific such as QSARs for amines and aldehydes [41, 42]. MultiCASE have numerous models for mutagenicity prediction and TOPKAT have Ames mutagenicity model. HazardExpert have comprehensive set of rules for mutagenicity prediction. (v) Like mutagenicity, carcinogenicity end points predictions are also more specific and many QSAR models exist in this area. The advantage of model specific chemical class is its simplicity and good predictive power in understanding the mechanism of action [43]. Modelling work have been carried out using multivariate data, from a heterogeneous database of compounds, and it is difficult to use due to lack of transparency in carcinogenicity data [44]. TOPKAT and MultiCASE contain FDA approved models for carcinogenicity predictions. OncoLogic which contains comprehensive collection of carcinogens, also has powerful models taken from US EPA [23]. QSARs of other modeling endpoints include development/ reproductive toxicity, acute and chronic environmental toxicity and bioaccumulation [14].

In silico toxicity prediction also include other related physicochemical and pharmacokinetics parameters such as solubility, fraction of drug absorbed, metabolism, affinity for efflux transporters, bioavailability, binding to plasma proteins, tissue:blood partition coefficients, blood-brain barrier (BBB) partition coefficient, volume of distribution, clearance and half life.

Simplest models were established using rule based screening of compounds by different research groups such as, Lipinski *et al.* [45], Egan *et al.* [46], Norinder and Haeberlein [47] and Veber *et al.* [48]. In 1997, Lipinski *et al.* published a guiding "rule of 5" simple method for drug screening, after analyzing absorption properties of 2287 compounds. They concluded that a drug show poor absorption if: (i) molecular weight exceeded 500, (ii) the sum of OH and NH hydrogen bond donors exceeded 5, (iii) the $\log K_{ow}$ exceeded 5 and (iv) the sum of N and O atoms acting as hydrogen bond acceptors exceeded 10. Other classic work for chemists include graphical representation whether the solubility of a compound is acceptable or unacceptable in terms of absorption of a medically effective oral dose of the drug [49]. The study of the prediction of the drug absorption using multivariate statistics by Egan *et al.* shown that compounds show poor absorption if polar surface area (PSA) is < 148.1 $Å^2$ and log K_{ow} above 5.88 [46]. Similarly simplistic models with few rules were proposed by Norinder and Haeberlein for the prediction of the blood brain barrier (BBB) partitioning i.e. the compound is likely to enter the brain if $[N + O] \leq 5$ and log BBB portioning is positive, if log K_{ow} $-(N + O)$ is positive. This have direct impact of the importance of the brain as a site of action of drugs and as a site of potentially serious toxic side-effects of drugs, which is designed to act elsewhere [47]. The study by Veber *et al.* shown the effect of molecular properties on oral bioavailability of drugs using simple classification system, where compounds with ≤10 rotatable bonds and a PSA of ≤ 140 $Å^2$ (or ≤ 12 hydrogen bond donors or acceptors) show good bioavailability [48]. All the above mentioned models will further improve by refinement, with more data became available.

Nowdays, QSAR and molecular modeling techniques are well established and great advancement were made in the prediction of the pharmacokinetic and toxic effects of compounds. High throughput screening and data mining approach in diverse fields are required and a coordinated approach needed for the production of the useful model. Failed drug candidates are also useful in order to

identify unfavourable characteristics in it. The physiological mechanism responsible for uptake, distribution, storage and elimination of the compound from the body are of main concern, and more robust models need to be developed for predicting the toxicokinetic fate of these chemicals.

Quality and Assessment of *In Silico* Toxicity Prediction Model

In silico model prediction, using different expert systems in toxicoinformatics, need assessment in terms of its sensitivity and specificity, which is also interrelated. An optimization of a model towards higher sensitivity values results in a reduced specificity. The sensitivity of the model is the ratio of correctly predicted toxic molecules to the total number of toxic molecules. Specificity refers to the ratio of correctly predicted non-toxic molecules to the total number of non-toxic molecules. Furthermore, an integrated parameter concordance (or accuracy) in assessing overall performance of these models also need attention which is the ratio of correctly predicted molecules to the total number of tested molecules. An overall model performance (Q)SARs for evaluation of the commercially available software for human health and environmental endpoints was performed by European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) and others [50, 51]. It is crucial to exercise the real predictive capability of different expert systems using molecules whose experimental results are not available. Two NTP comparative exercise on the prediction of rodent carcinogenicity have been analysed (e.g. sensitivity and specificity) by various research groups [43]. This exercise is found to be very important for judging the real capability of predicting the carcinogenicity of untested chemicals. A maximum accuracy of 65-70% attained for noncongeneric chemicals in most of the expert systems, which is encouraging. Classical QSAR methods are also satisfying for individual classes of carcinogens and mutagens with 80-90% accurate [52]. It should also be noted that the applicability domain of a QSAR system is to estimate the similarity of the predicted compound to the compounds used in the training set, which is however, always descriptor dependent for that specific case. Ideally, the descriptors used for estimation of the applicability domain for the model development should be mechanistically related to the predicted endpoint [53].

CONCLUSIONS

Computational toxicology is now widely used for lead chemical development, and are capable of providing valuable information in drug discovery process. These *in silico* toxicology experiments can play a major role in decreasing time to market, reducing animal experiments, assessing late stage attrition, and strategic planning of pharmaceutical and chemical development processes. Good predictive models for toxicity parameters depend crucially on selecting the right mathematical approach, the right molecular descriptors for the particular toxicity endpoint, and a sufficiently large set of experimental data relating to this endpoint for the validation of the model. In the next 10 years or so, the degree of automation of *in silico* modelling and data interpretation will continue to increase with the integration of medium- to high-throughput *in vitro* and *in vivo* assays to reduce the risk of late-stage attrition, and second, to optimize the screening and testing by looking at only the most promising molecules.

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Received: 26 September, 2006 Revised: 16 November, 2006 Accepted: 17 November, 2006