

# Editorial

## ***In Silico* Toxicology Challenges for Pharmaceuticals: Complacency or Controversy?**

Many areas of toxicology are changing. Various initiatives, such as the REACH system in the European Union and 'Tox21' in the United States, are forcing scientists to change their attitudes and move away from what they regard as being comfortable. Within the pharmaceutical industry, change in the field of toxicology is polarised: at one end of the spectrum, change is fast, as cheap and rapid screens are sought to eliminate toxic candidate drugs; at the other end of the spectrum, change is slow, as the whole range of traditional animal testing is still required to provide evidence for regulatory submission. There is inevitable historical scepticism and doubt over the use of non-traditional tests in assessing the toxicity of pharmaceuticals. However, it is hoped that such scepticism is declining, as there is increasing acceptance of non-animal test methods in many areas associated with the identification, development and optimisation of new chemical entities. The papers in this special edition of *ATLA* describe the current state-of-the-art, and raise some questions over whether we are complacently walking into an era of *in silico* toxicity assessment for pharmaceuticals which will result in problems later on, or whether this is too controversial an issue to be tackled with our current understanding.

*In silico* methods form a valuable part of the battery of techniques available to act as alternatives to traditional toxicity testing. *In silico* methods for toxicology cover many approaches, from (quantitative) structure–activity relationships ([Q]SARs), category formation and read-across, through to the database compilation and integration of workflows. They are particularly useful in the identification of a number of classic toxicities, for example, mutagenicity, but they are also able to react rapidly to new problems, such as cardiotoxicity. The types of techniques employed vary, from the simplistic to the very complex.

It is important to appreciate that there are many opportunities to use these techniques within the drug discovery and development pipelines. Crude and rapid approaches are likely to be used as screens for large numbers of compounds early in the discovery process. These are used routinely to identify toxic or non-soluble compounds. Many are

based on trivial calculations of molecular structure (cf. the 'success' and widespread application of the Lipinski rules in assessing oral absorption), and are capable of rapidly screening millions of structures. At the other end of the drug development process, more-detailed approaches (e.g. receptor modelling) can be applied to a small number of molecules. These studies are labour intensive and computationally expensive, but will assist in the optimisation of drugs to eliminate toxicity. Many of these techniques are routinely applied across the industry.

In order to reduce controversy in toxicity prediction and to increase confidence and acceptance, the method used to predict toxicity should reflect the complexity and type of endpoint. The user must have the expertise and courage to dismiss trivial or overfitted models for complex endpoints as being inadequate, and to search for better solutions. Thus, for an interaction with DNA, a structural alert for covalent binding may suffice. For a receptor-mediated toxicity, the interaction between ligand and receptor may need to be addressed, requiring a full and time-consuming assessment. Above all, the desire for global correlations and endlessly improved statistical fits is not necessarily a good thing. Modellers must appreciate the errors and limitations of the data they are modelling. Good examples of where we must improve our thinking are in the modelling of chronic toxicity and reproductive toxicity. For instance, the modelling of a No Observed Effect Concentration (NOEC) for a chronic toxicity is a very complex process. This value represents the level at a particular organ. Understanding that NOEC values cannot necessarily be compared across organs will be of great benefit. It is these types of endpoints where (at least at the initial stages) category formation may be useful to build groupings of molecules. It is also easy for the modeller to be caught up in the intricacies of modelling and forget the effect of biological error and variability. Conversely, biological error or the precision of a test system should not be used as an excuse for the inadequacies of a predictive model!

*In silico* toxicology has progressed in the last five years. However, there is seemingly little overlap

between activities in the pharmaceutical industry and in other areas — for example, in the case of the REACH system, there is much that could be transferred both ways. As new tools and techniques are developed as a reaction to it, these could provide solutions within the pharmaceutical industry. The obvious proviso to this statement is that models developed for fine chemicals must be used with caution for pharmaceuticals (these compounds will be outside of the applicability domain of models for fine chemicals, so they must be carefully evaluated). This should, of course, be a two-way process — industries assessing fine chemicals can take the knowledge and expertise from the pharmaceutical industry to solve their problems. The pharmaceutical industry has the capabilities/resources and the need to solve some ongoing problems in *in silico* toxicology. The key will be the possibility of sharing this expertise, for the benefit of society as well as the pharmaceutical industry as a whole, rather than confining the benefit to individual companies. Whilst encouraging this process, we must be realistic in our expectations of transfer/release of knowledge, as this is an industry that relies on defending its intellectual property.

One area where cross-over between different sectors of industry could be encouraged is mode of action category formation. This will require knowledge of mode and mechanism of action, and how to form the category. Category formation is a simple and powerful technique. It is transparent and ideally based on mechanism of action. For success in this area, we need not be restricted by current approaches to forming categories, such as 2-D structural alerts, but should use our imagination and the full range of molecular modelling tools to further our understanding of the compound's chemistry, receptor binding, and pharmacophore identification, etc. These require a number of pieces of information, in particular: a) an understanding of the mode or mechanism of action; b) tools to translate mechanisms into usable computational tools; and c) data to fill the categories to enable read-across. Increased effort should be invested in transferring the powerful computational tools used in drug design to the solution of toxicological problems.

*In silico* toxicology is increasingly being seen as an integrated science. The integration is both intra-*in silico* (using a battery of approaches to obtain a consensus) and inter-*in silico* (integrating with other [non-]test information, such as *in vitro* and *in chemico* data). *In silico* estimates of toxicity should be combined with an understanding of how toxicokinetics can affect the endpoint. The real power of *in silico* predictions comes, not from individual assessments or expecting a one-to-one replacement, but from accepting the prediction as a

piece of information that will have an associated confidence level. Combining concordant information (whether *in silico* or other) increases confidence.

*In silico* modelling is usually a data-hungry activity. There will always be a desire for more data to be released. Those outside the pharmaceutical industry have often looked jealously at the data that 'must' be available to pharmaceutical industries. It is naïve to assume that these industries will release current or historic data, especially those that give their business a competitive edge — however that may be measured. Other practical problems include the physical retrieval of historical data, and the fact that (with the exception of regulatory methods) many tests will have been performed by using non-standard methods — a fact which must be appropriately addressed. One possible starting point is to collect the 'standard' data available for drugs. Data can be compiled, but often there is a lack of references to the original tests or studies, but merely to the presence or absence of a particular effect. One initiative could be for pharmaceutical industries to compile the toxicity profiles into a suitable data format, with meta information included for general or in-house use. Such pharmaceutical data could have a number of applications: a) they can be used to develop new models or categories of toxic compounds; b) they could be used to test the domains and suitability of current models; and c) they could also provide a template of how data should be stored in future experiments.

As well as obtaining the data, decisions must be taken, industry-wide and rapidly, to ensure that the data are stored in a usable and future-proof format. The storage of data is more complex than simply associating a number with a chemical name in an electronic spreadsheet. Among the complications are how to record chemical structure (i.e. 2-D, 3-D, identification of stereoisomers, etc.), and how to record the method and the level of detail required. Also, there will be technological issues of how to ensure compatibility with other software, both now and in the future, as well as the safe storage of non-public data to enable their use alongside public data.

This is not to say that data retrieval and compilation activities are not being undertaken: the possibilities of data mining have allowed the compilation of numerous databases. For instance, the US Food and Drug Administration has initiated collaborations with a number of companies (e.g. Leadscope Inc., Lhasa Ltd) to provide these data. Other data compilation activities include WOMBAT (Sunset Molecular Inc.) to pull out data from literature sources. The last few months have also seen a number of data searching facilities

crossing over databases and resources (e.g. the US Environmental Protection Agency's ACToR, the OECD's eChemPortal, and the European Commission's European Chemical Substances Information System [ESIS]). Such resources are likely to be of great value — however, they pull together information from disparate sources of varying quality, so strategies are needed to determine their appropriate use.

As well as existing data for drugs on the market, there is also the intriguing possibility of using data related to 'failed' drugs. This is important, as drugs that reach the marketplace are inherently 'acceptable for use' and 'bioavailable', hence the data associated with them is for such 'successful' compounds (and thus the data on drug failures are omitted). This skews data sets for modelling toward the more successful areas of chemistry. Releasing more information from drug failures would greatly expand our knowledge. Such concepts bring into focus how this could be achieved. Data could be released publicly or, should the release of structures be considered problematic, through a safe broker — such that useful knowledge could be obtained without the release of full chemical structures.

In terms of other types of modelling, e.g. physiologically-based pharmacokinetic (PBPK) models, real progress can only be made when there is a greater availability of human *in vivo* data (PK, efficacy, etc.) that are not currently in the public domain. These will be data that have been generated in clinical trials, many of which will have resulted in drugs not making it to the market for a variety of reasons. This is a potentially great resource of *in vivo* human data. In addition, such data would assist in the development of better designed *in vitro* assays and experiments, as well as enabling better *in vitro*–*in vivo* scaling.

The ultimate challenge for the *in silico* toxicology of pharmaceuticals will be regulatory acceptance. This means that we must assume that the predicted value will be acceptable to regulatory agencies worldwide. This may require a framework to achieve this. Despite their name, the *OECD Principles for the Validation of (Q)SARs* will not allow for the formal validation and acceptance of *in silico* models. They should be thought of as a framework to describe, characterise and evaluate a model, rather than as a formal validation process (as applied to *in vitro* methods). In addition, these principles were not formulated with pharmaceuticals in mind, so they may require updating or amendment. Thus, a rational and open dialogue must be developed between regulators, industry and modellers. There is no point in expending resources to attempt to solve problems which will have no reg-

ulatory acceptability. To achieve acceptability, regulators should be encouraged to state what will be required to make an *in silico* prediction acceptable.

Other challenges abound for pharmaceuticals. Idiosyncratic drug toxicity is likely to be one of most difficult problems to solve, if it is not addressed in a fundamental and mechanistic manner. The prediction of metabolites (reactive or not) remains very difficult, beyond a simple identification of 'likely metabolites'. Models are required to predict the stability (and activity) of metabolites, as well as the likelihood of their being present. Strategies are also required for the use of this information. Also challenging to *in silico* toxicology is the issue of modelling of mixtures. The significance of this to pharmaceuticals needs to be addressed.

There is little complacency over the use of *in silico* toxicology in predicting the effects of pharmaceuticals. There is, however, a lot of potential controversy. Progress will only be made by addressing the controversial issues and building rationally on the current successes to face our challenges. Among these challenges are: building the databases for endpoints; the employment of suitable modelling approaches for these complex endpoints and idiosyncratic drug toxicity; integrating across models, approaches and chemistries; and the formation of categories or groupings of compounds to address these approaches. Ultimately, it will require courage to use these approaches at the regulatory level and gain regulatory acceptance. This is not a problem that will be solved by one individual or business — it should be tackled across the industry. The desire is for freely available tools — however, this must be realistic, as directed funding will still be required for their initial development and their ongoing maintenance. This will require an initial and ongoing outlay. One excellent and exciting approach is the new EU 7th Framework Programme Project, 'eTox', funded through the Innovative Medicines Initiative (IMI) scheme, which aims to develop predictive *in silico* technologies for the toxicity of pharmaceuticals.

This special edition of *ATLA* has brought together a series of articles that illustrate various aspects of progress in different areas of the pharmaceutical industry, academia and regulatory agencies. In editing these articles for the special edition of *ATLA*, I see little complacency but plenty of controversy, both now and in the future. However, I positively welcome and encourage the controversy, as it will stimulate future efforts and will lead to solutions to the problems.

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*Mark T.D. Cronin*  
*School of Pharmacy and Chemistry*  
*Liverpool John Moores University*  
*Byrom Street*  
*Liverpool L3 3AF*  
*UK*  
*E-mail: m.t.cronin@ljmu.ac.uk*