Hail Caesar

Mathematical models for toxicity testing promise to reduce the number of animal experiments and costs associated with REACH compliance, says Nick Price

From the publication of the European Commission White Paper *Strategy for a future chemicals policy* in 2001, to the first implementation in June 2007, Europe's REACH regulations have been the subject of heated debate. A general consensus has emerged that full implementation of REACH would require testing of around 30 000 existing substances, cost more than €2bn, and use an additional 10-22m test animals, if no alternatives to animal testing were available.

Simultaneously with the development of REACH, the EU has been developing its policy of replacing reducing, and refining the use of animal tests: the so-called 3Rs policy. Among the likely alternatives are Quantitative Structure-Activity Relationships (QSARs), which express the mathematical relationship between the biological activity of a series of chemical compounds and their physicochemical properties. Carrying out a QSAR involves obtaining 2D or 3D structural co-ordinates for each chemical in a toxicological dataset, calculating a range of molecular descriptors (properties), and subjecting the data to mathematical modeling to obtain a relationship between the descriptors and the measured biological activity.

Used for many years in the laboratory, until recently QSARs were unlikely to be acceptable in a regulatory context for a number of reasons. Most QSARs were developed for a narrow range of closely related molecules, and were unlikely to be relevant for chemicals falling under REACH. Few QSARs have

In Brief

- **Mathematical models called QSARs promise to reduce the number of animal tests for REACH**
- **Collectively, they should save an estimated €700-900m in testing fees**
- **The EU's project CAESAR aims to make such models freely available on the internet**
- **Models for five key toxicological endpoints will be available by mid-2009**

been developed for toxicological endpoints required under REACH. Also, since there were no agreed methodologies for developing QSARs, validation would be a problem.

But conditions now appear to be favourable for the use of QSARs in REACH. The massive increase in computing power in recent years, coupled with the increased availability of good quality toxicological datasets, has made the computational chemistry aspects of constructing QSARs much more amenable, and the advent of data mining and machine learning has opened up possibilities for developing QSARs on more heterogeneous data sets. With regard to validation and methodology, a document by the Organisation for Economic Co-operation and Development (OECD) gives very comprehensive guidance on what is considered acceptable for a QSAR to be used in a regulatory context (see Box).

Compared with an estimated cost of more than £1m for a single rodent carcinogenicity study, the use of a validated QSAR from CAESAR will be free and take a matter of minutes

Enter CAESAR - Computer Assisted Evaluation of industrial chemical Substances According to Regulations - which aims to develop robust, validated QSARs specifically for REACH, and to provide the models free of charge on the internet.

A 6th Framework Programme research project, the idea for CAESAR emerged from an earlier EU project, DEMETRA, which developed QSARs for pesticides (http://www.demetra-tox.net/) for use by those involved in the pesticide industry or its regulation. This approach seemed ideal for REACH chemicals and so CAESAR was born.

The selection of endpoints was a key step. From the perspective of saving animal lives and costs, it was important to select endpoints that were the most resource-intensive in conventional tests. According to OECD figures, the most resource-intensive tests are two generation reprotoxicity tests, developmental toxicity, mutagenicity, skin sensitisation, bioaccumulation and carcinogenicity.

Applying QSARs to replace these tests should result in massive cost savings. Compared with an estimated cost of more than £1m for a single rodent carcinogenicity study, for example, the use of a validated QSAR from CAESAR will be free and take a matter of minutes.

One of the key lessons learned in DEMETRA and applied in CAESAR was that quality assurance at all stages of the QSAR process is vital. Built into the CAESAR workplan is a series of steps involving checking and cross checking of data by two or more partners. CAESAR has partners with expertise in biochemistry, toxicology, computational chemistry and data mining, and the advantages of having a good spread of all the expertises required soon became apparent.

Currently, biological data from animal or cell experiments are used for regulatory purposes but often published datasets can be of low quality or have high variability. The project team found that, even with high quality biological data, the variation in individual mean data points could be 15% or higher, and in many cases only a single determination was available for particular chemicals. Data used in the CAESAR project were selected on the basis of quality, availability and the presence in the dataset of structural information. It was not possible to use molecular modelling to build every structure from scratch and so datasets in the structure data file (sdf) format were favoured as they contain two-dimensional structural co-ordinates.

A further major step in the early part of the project was the quality assurance of the structural data. In cross-checking the many thousands of structures using desktop molecular modelling software, many errors were found in the published data files as well as, occasionally, in proprietary 'chemical finder' websites. Datasets had to be 'cleaned' of all incorrect structures, ambiguous or mixed structures, and those containing inorganic elements, cross-checked by at least two partners and then submitted to partners for descriptor calculation and mathematical modelling.

A range of mathematical techniques are used, including linear regression, neural networks, self-organising maps, genetic algorithms, and machine learning.

Models that have acceptable predictive powers, based on a range of validation tools, are incorporated into larger 'hybrid models' that will be the final output of the project in mid-2009. At the current stage a number of good models are emerging. The project team is confident that it will succeed in its aims, and anticipates that a set of models for developmental toxicity, mutagenicity, skin sensitisation, bioaccumulation and carcinogenicity, valid for a wide range of chemical types, will be submitted to the European Chemicals Bureau for validation for use within REACH.

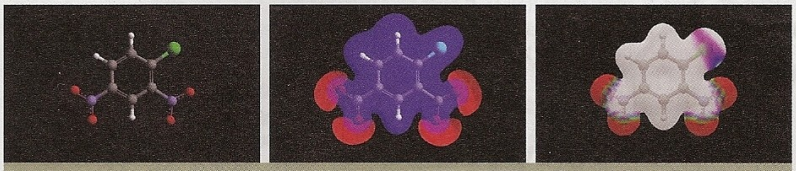
REACH requires that all available evidence is used in the assessment of a chemical. Although QSAR will never provide the complete answer, it will undoubtedly be used as part of the 'body of evidence' for a wide range of chemicals, especially in the early years of REACH when submissions will rely heavily on non-animal data.

Nick Price is one half of the independent consultancy Technology for Growth, and a fellow of the UK Central Science Laboratory.

Regulatory issues

To be useful for regulatory purposes, the OECD guidelines suggest that QSARs should have:

1. **A defined endpoint; the QSAR must relate to a toxicological parameter used in REACH as an indicator of safety.**
2. **An unambiguous algorithm; the model must have been generated by a specific definable and repeatable mathematical process.**
3. **A defined domain of applicability; types of chemicals for which the model is valid must be stated.**
4. **Appropriate measures of goodness-of-fit, robustness and predictability; in addition to statistical tests for validation, it is now required that an external test set be applied and the resulting accuracy of prediction specified.**
5. **A mechanistic interpretation, if possible; an understanding of how the model works at the biological level is useful in establishing its validity.**



Skin sensitiser: 1-chloro, 2,4-dinitrobenzene, one compound being modelled by CAESAR

Project partners

CAESAR (www.caesar-project.eu/) is led by Emilio Benfenati of the Istituto di Richerche Farmacologiche Mario Negi in Milan, Italy. The other partners in the project are: the Central Science Laboratory, (UK), Biochemics Consulting, (France), Polytecnico di Milano, (Italy), Knowledge Miner Software, (Germany), Liverpool John Moores University, (UK), UFZ, (Germany), Kemijski Inštitut, (Slovenia) and TNO, (Netherlands). In addition, CAESAR has a formal advisory board which includes representatives from the chemical industry and from the EU regulatory authorities.