



MAMMALIAN TOXICOLOGY – PROPERTY PREDICTION AND QSAR TECHNIQUES

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Date: February 2004

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1. Introduction

There are a number of reasons to use alternatives to animal testing wherever this is possible within the law. The proposed EU regulatory system REACH (Registration, Evaluation and Assessment of Chemicals), has stimulated interest in the use of methods that reduce the amount of animal testing because of the enormous quantity of toxicological data that may be required. This requirement cannot realistically be met quickly by animal testing, and minimising animal testing is one of the aims of REACH. Of the other reasons, those most compelling to the chemical industry are economic considerations and the pressure of public opinion.

The use of QSAR (Quantitative Structure Activity Relationships) in ecotoxicology is well established, and predictions can be made with a sufficient degree of accuracy. The situation in mammalian toxicology is rather different. There are a number of QSAR systems available, which allow prediction of a wide range of biological endpoints. However, their limitations mean that they are not universally applicable, and considerable expertise is needed in their use. "Expert systems" attempt to overcome this problem by incorporating QSARs into computer programs that enable less expert users to access them. Prediction of toxicity by QSARs and expert systems can be valuable in the selection of lead substances, and in prioritisation for testing. However, some expert knowledge is still required to evaluate the resulting information, particularly to ensure compliance with current regulations.

Whatever prediction methods are used, they must be understood sufficiently to know if a valid prediction can be made for the substance under consideration. For substances that are relatively simple and well known, QSARs are widely used and valuable. Extrapolation of prediction to substances that are very different from those used in developing the QSAR or expert system may not produce valid results, so novel structures are quite possibly outside the scope of valid methods. This review looks at the methods available, the endpoints to which they have been applied and the range of chemical classes to which they are relevant. Some familiarity with the general area is assumed.

2. QSAR

The basic principle of QSAR is that the molecular structure of a chemical influences its physicochemical properties and biological activities¹. If the biological activity and some aspect of structure can be expressed numerically, various mathematical techniques are used to link them. There is therefore the theoretical potential to predict biological activities from chemical structure. The use of QSAR to calculate physicochemical properties is well established. The potential use of QSAR in predicting mammalian toxicity has been

recognized for some time, as both cost and ethical considerations make animal testing less attractive^{2,3,4}. In addition, animals are not necessarily good models for toxicity, as some toxic effects show species specificity (e.g. non-mutagenic carcinogenicity⁵). The complexity of toxicity in animals has led to the development of expert systems (see below).

3. Expert Systems

Expert systems (usually computer based) provide access to predictions of the toxicity of chemicals using experimental data and/or rules derived from data¹⁰. Most use a combination of logic trees and QSAR to estimate a wide range of toxicological endpoints. The approach of expert systems is either statistical (e.g. CASE, TOPKAT) or knowledge based (DEREK, OncoLogic)⁸. There have been divergent opinions of the usefulness of expert systems: in 1995 Cronin and Dearden⁶ concluded that although some systems are well advanced in certain areas, their reliability needs to be increased. In 1997 Taningher¹⁰ contrasted Benigni's opinion, that much experimentation is needed for even approximate assessment of chemicals to be possible, with Ashby and Tennant's view that prioritisation of chemicals (for testing) has been achieved. Their view is almost certainly true; these contrasting views probably represent the level of accuracy the reviewers are hoping to achieve.

A comparison of four expert systems was undertaken by Richard⁸. Both DEREK and CASE identify toxicophores (or biophores) which are functional groups that are statistically likely to be responsible for the toxicological endpoint in question. This can be misleading as metabolic transformation may modify these groups, and other functional groups may modify the effect of the toxicophore. Expert systems are being improved all the time and they provide valuable access to expert knowledge. However, there is still a need for expertise in assessing the reliability of the predictions that they make, and their applicability to regulatory criteria.

4. Techniques available

The development of a QSAR requires firstly toxicity data for a group of chemicals (the training set), secondly property data for those chemicals, and then tools of statistical analysis to relate these two. Property data may be experimentally determined, calculated or modelled. Techniques such as principal component analysis are used to find the most important variables, or combination of them, in multi-dimensional datasets. The result of the statistical analysis will be an equation derived by methods such as multiple linear regression, or a non-linear computational neural network (CNN) (also known as an artificial neural network (ANN)). These results are validated using appropriate statistical techniques (most commonly the value of r^2 is quoted, indicating the ability of the model to reproduce the data in the training set). The predictive power of the model must also be tested, either by applying it to a further set of chemicals with known properties (the prediction set), or by internal validation.

Properties used in QSAR include physicochemical parameters such as the octanol-water partition coefficient, log P, and critical micelle concentration, CMC. Other properties used are topological, such as atom counts, molecular weight and connectivity indices. Electronic parameters including HOMO (highest occupied molecular orbital) and LUMO (lowest

unoccupied molecular orbital) energies and dipole moments, and geometric descriptors e.g. inertia, molecular volume and surface area, are also used. Other properties are modelled such as membrane interactions²³ and the EVA descriptor used by Livingstone⁹.

The toxicity data currently in use are generally traditional measures of toxicity such as LD₅₀. Schultz and Seward²⁶ suggest that further progress in the prediction of biological endpoints will depend on the development of surrogate endpoints in place of whole animal toxicity testing. Data from these could be used to derive QSARs that could then be incorporated into expert systems that include software to predict metabolite formation.

4.1 Acute toxicity modelling

There are few models for acute toxicity, because of the wide variation in quality, source, accuracy and organism used in the data, and poor understanding of the mechanisms involved. Acute toxicity is a whole body phenomenon, with a wide variety of possible mechanisms, and prediction requires knowledge of metabolism, bioaccumulation and excretion. So most studies have been done on limited ranges of chemicals. Prediction of acute toxicity by the expert system TOPKAT predicts only 50% of chemicals to within a factor of two¹⁴.

4.2 Carcinogenicity prediction

A lot of work has been done on the prediction of mutagenicity and carcinogenicity with limited success. These are endpoints that are very hard to define, as there are a variety of mechanisms of carcinogenicity. Many carcinogens are mutagenic, forming covalent bonds with DNA, so mutagens can be predicted by identifying electrophilic functional groups. In some chemical classes, metabolic transformations that are usually detoxifying instead activate the chemical, producing a potential carcinogen (e.g. the N-oxidation of aromatic amines¹⁷). Other carcinogens are promoters, stimulating cell proliferation. Promoters are much more difficult to predict, as they are heterogeneous structures with diverse activities, and many are species specific.

4.3 Modelling of eye irritation

This is another area that has attracted a lot of attention, for ethical reasons. The traditional method of evaluating eye irritation is to apply the substance under consideration to rabbits' eyes, and to determine the effects on the eye, producing a single value known as the Draize Eye Score (DES). The results of QSAR analysis of eye irritation for several groups of chemicals illustrate the problem of the reliability of data for biological endpoints. The results are not statistically robust, with r^2 values of 0.7 – 0.78, but these values reflect the variability in the data used, and so it may not be possible to predict eye irritation with great accuracy.

5. Endpoints covered

Published QSARs cover acute toxicity, carcinogenicity, mutagenicity, skin irritation, respiratory irritation and eye irritation. There is considerable variation in the usefulness of these QSARs, both in terms of statistical robustness and range of chemicals to which they are applicable. The following table summarises the QSARs described in the papers listed in the references.

Endpoint	Description	Adequacy	Reference
Acute toxicity (oral)	ANN model for diverse substituted anilines	Reasonable 92 compound T-set, 12 compound P-set, 5 descriptors	11 Johnson and Jurs
Acute toxicity (oral)	QSAR for procaine analogues	Poor 15 compounds 2 descriptors	12 Amaral <i>et al</i>
Acute toxicity (oral)	Rule-based QSAR for diverse alcohols	Not true QSAR. 95 alcohols in T-set. 25 in P-set. 88% correct prediction rate 6 descriptors	13 Guilian and Naiban
Acute toxicity (oral)	QSAR for ketones	Reasonable 13 ketones 1 descriptor	14 Cronin and Dearden (2) (Lipnick)
Acute toxicity (oral)	QSAR for anilines	Reasonable 29 substituted anilines 2 descriptors	14 Cronin and Dearden (2) (Jackel and Klein)
Acute toxicity (oral)	Model for heterogeneous chemicals (TOPKAT)	50% out of 2066 predicted within factor of 2	14 Cronin and Dearden (2) (Enslin)
Carcinogenicity	ANN model for aromatic N compounds	Poor 92 compounds 13 descriptors	16 Gini <i>et al</i>
Mutagenicity	QSAR for frame-shift mutations in aromatic amines	19 compounds 3 descriptors	17 Benigni (Trief)
Mutagenicity	QSAR for base-pair mutations in aromatic amines	19 compounds 3 descriptors	17 Benigni (Trief)
Mutagenicity	QSAR for frame-shift mutations in heteroaromatic amines	Poor 14 compounds 1 descriptor	17 Benigni (Ford and Griffin)
Mutagenicity	QSAR for base-pair mutations in QSAR for heteroaromatic amines	Poor 13 compounds 1 descriptor	17 Benigni (Ford and Griffin)
Mutagenicity	QSAR for mutations in	Poor	17 Benigni (Ford and

Endpoint	Description	Adequacy	Reference
	heteroaromatic amines	6 compounds 1 descriptor	Griffin)
Mutagenicity	QSAR for frame shift mutations in aromatic and heteroaromatic amines	Reasonable 88 compounds 4 descriptors	17 Benigni (Debnath)
Mutagenicity	QSAR for base-pair mutations in aromatic and heteroaromatic amines	Reasonable 67 compounds 3 descriptors	17 Benigni (Debnath)
Carcinogenicity	QSAR for carcinogenicity in mice of nonheterocyclic aromatic mono-amines	Poor 17 compounds 5 descriptors	17 Benigni
Carcinogenicity	QSAR for carcinogenicity in mice of nonheterocyclic aromatic amines (>1amino)	Poor 20 compounds 4 descriptors	17 Benigni
Carcinogenicity	QSAR for carcinogenicity in rats of nonheterocyclic aromatic mono-amines	Poor 20 compounds 5 descriptors	17 Benigni
Carcinogenicity	QSAR for carcinogenicity in rats of nonheterocyclic aromatic amines (>1amino)	Poor 20 compounds 4 descriptors	17 Benigni
Mutagenicity	QSAR for diverse compounds	Reasonable 90 compounds continuous descriptor	18 Livingstone
Respiratory irritation	QSAR for unreactive compounds	Good 42 compounds 1 descriptor	19 Cronin and Dearden (4)(Roberts)
Respiratory irritation	QSAR for unreactive compounds	Reasonable 39 compounds 3 descriptors	19 Cronin and Dearden (4)(Abraham)
Eye irritation	ANN model for neutral organic compounds	Reasonable 57 compounds 4 variables	20 Barratt
Eye irritation	QSAR for diverse liquids	Reasonable 38 compounds 4 descriptors	21 Abraham <i>et al</i>
Eye irritation	ANN model for cationic surfactants	29 tests on 19 compounds 4 variables	22 Patlewicz <i>et al</i>
Eye irritation	MI-QSAR for diverse chemicals	38 compounds 5 descriptors	23 Kulkarni <i>et al</i>

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