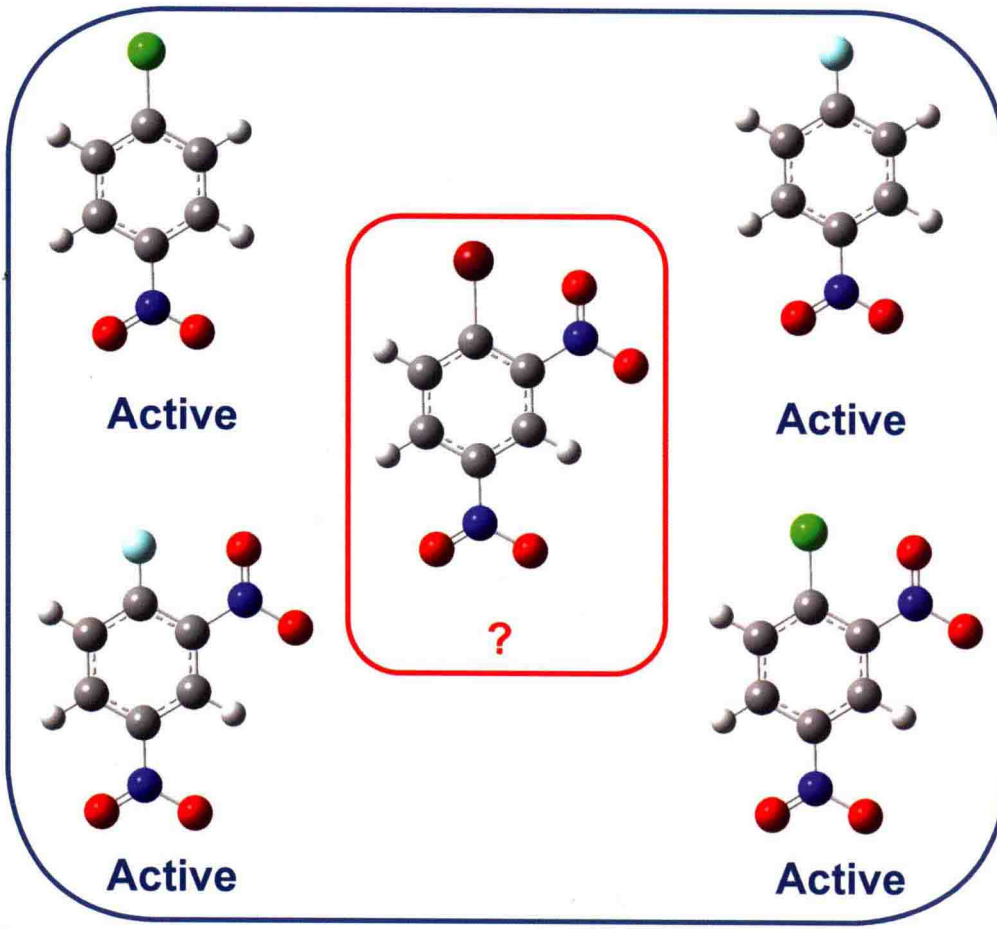
Issues in Toxicology

Mark T D Cronin, Judith C Madden, Steven J Enoch
and David W Roberts

Chemical Toxicity Prediction

Category Formation and Read-Across



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Preface

To address the societal problem of assessing the safety of hundreds of thousands of chemicals, without resorting to extensive animal testing, a paradigm shift was required in the way in which toxicity data were obtained. Computational tools to predict toxicity have been available for over 50 years, but there has previously been a reluctance to accept predictions from these models, particularly for regulatory purposes, with lack of model transparency often being identified as an issue. However, we are now in an era where toxicologists and computational modellers work together much more closely to resolve the problems posed in predictive toxicology, i.e. how to ensure the safety of chemicals for man and the environment. The mutual benefits of combining research efforts from diverse areas is now apparent and real progress is being made in both model development and acceptance.

This is a rapidly expanding field and the past few years have seen an increase in the numbers of publications in toxicology and the main toxicological conferences now routinely include modelling. There is also an increasing trend to see modelling at the heart of a toxicological study or project, e.g. to help identify chemicals to test, or to rationalise the results. Computational modelling has similarly benefitted from the input of toxicological expertise, for example in the development of consistent ontologies for new databases and the identification of key (modellable) steps within a toxicity pathway.

There are many well documented reasons for this shift in the way in which models are developed and used, not least the pressures of having to find solutions for European, and other, legislations e.g. REACH, the Cosmetics Regulation and others. Ethical pressures, financial and logistical constraints (not all the chemicals can be tested) as well as the adoption of 21st Century Toxicology, with the ultimate goal of moving away from the old animal-based paradigm of testing, have all played a role.

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This book addresses a specific area of computational toxicology that has seen remarkable growth in the past five years, namely the grouping of molecules – the so-called formation of categories – to allow for toxicity prediction from read-across. The general grouping approach has been shown to be transparent and easy to perform, making the process more accessible to toxicologists and more amenable for use in regulatory submissions. Whilst product development is not the primary aim of this technique, some of the information will be useful for designing safer new products or replacing existing toxic compounds with those that may be more benign. These new developments mean that for certain scenarios, i.e. well characterised chemicals and simpler endpoints, it could be argued that there is now little information that could be gained from testing that is not easily predicted from read-across; therefore obviating the need for *in vivo* testing for these compounds. For the more challenging endpoints, the situation is different and more work is required, not least identifying the key steps within the pathways, gathering and modelling data. Recently there has been a drive to develop a framework for organising the chemical and biological interactions that result in toxicity. This has led to the development of the Adverse Outcome Pathway approach, an important component of which is the identification of key steps that are amenable to modelling, e.g. by using read-across.

This book provides the background to the process of grouping for the purpose of read-across for toxicity prediction. It provides practical solutions for those wishing to perform read-across and identifies where more research and collaboration are needed and how this could be achieved. The concept of using this information within an Adverse Outcome Pathway is also described providing a framework for organising and using new information as it is generated. In Europe, over the time period in which this book was being written, (quite a long time as it turned out!) there have been several cycles of REACH submissions and the Cosmetics Regulation has been implemented. This has not only changed the way in which read-across for toxicity is perceived and utilised, but has also resulted in useful guidance, case studies and (often heated) debate on the subject. We have been fortunate that we have been guided by much high quality work from industry, academics, various parts of the European Commission, as well as the Organisation for Economic Co-operation and Development (OECD), not forgetting the Non Governmental Organisations. This book brings together the expertise developed recently in this area, providing greater insight and details on the process and application of read-across and its potential in developing Adverse Outcome Pathways. A history of the science, recent developments, practical, technical guidance and a philosophy for future developments are all presented.

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CHAPTER 1

An Introduction to Chemical Grouping, Categories and Read-Across to Predict Toxicity

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1.1 Introduction – Ensuring the Safety of Exposure to Chemicals

Modern society requires safe chemicals. However, nothing is without risk and there is increasing pressure to identify hazardous chemicals and replace them with those that are more benign. In order to ensure the well-being of their population and the environment, governments enforce legislation to determine the effects of chemicals and ensure that every day accidental or occupational exposure will not cause harm. This is desirable for all substances that man comes into contact with, or that may be released into the environment, whether the substance is in foods, medicines, pesticides, fertilisers, or cosmetic ingredients (amongst many other types of chemicals that are in use). Different regulations are applicable to each type of chemical associated with a particular use.

In order to determine the risk associated with the use of a chemical, a certain amount of information is required. Firstly, a means of defining risk is a pre-requisite. In this context, risk is a function of the intrinsic hazard of a chemical

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and the exposure. Considering hazard, this can be considered as the ability to cause harm to a species, be that organisms that are deliberately exposed to the chemical or a non-target (for instance, environmental) species. Exposure can be simplistically considered as the quantity of a chemical to which the target and non-target species are exposed.[1]

Within the current definition of risk, information is required regarding the hazards of chemicals; this is provided by the science of toxicology. Assessing the toxicity of chemicals involves determining what the harmful effects of a chemical may be, *i.e.* toxicity to particular organs, effects to the skin, lethality, tumour promotion and countless others. Assessment normally involves testing for these effects and being able to use the test results in a manner that is protective of man and the environment. The tests and the information they provide need to be scientifically credible and satisfy the needs of the manufacturer, government or regulatory agency that has to interpret them and, ultimately, the user or consumer for whom safety must be assured. The information must be reliable, trustworthy and protective, *i.e.* precautionary. The need to determine the hazard of a chemical has resulted in the toxicological testing of chemicals for a wide range of specific effects, *e.g.* the ability to promote tumours. These effects can then be reported and interpreted to identify hazard. The most accepted paradigm for the identification of the majority of toxic effects has been the use of animal testing, through a series of standardised assays. However, the use of animals to identify hazard has received much criticism as being unethical, difficult to extrapolate results and findings to humans, costly and not always capable of identifying subtle or idiosyncratic toxicities.[2] Therefore, for decades, alternatives to animal testing have been sought. Amongst these are the so-called computational, or *in silico*, models which attempt to draw conclusions regarding the toxicity of a chemical from existing knowledge and/or its chemical structure. It is a selection of these techniques, those involving grouping similar chemicals together and reading across (or interpolating) activity, that form the focus of this volume.

With regard to exposure, a number of issues must be considered. The first is how much of the material is the organism in question exposed to? Also of importance is the time period over which the organism will be exposed, the route and manner of administration, *i.e.* the formulation (that may affect uptake). Consideration must also be given to whether local, *i.e.* at the site of exposure (if applicable), or systemic effects are of concern. Assessment of exposure therefore requires appreciation of uptake and bioavailability within the organism. A key principle to remember is that if there is no exposure to a chemical, or it is at a level below that which can cause harm (as defined by the toxicological assessment), there will be no risk.

In silico or computational toxicity prediction methods cover a very wide range of techniques and approaches, some of which are described in Sections 1.1.1 and 1.1.2. However, the main focus of this volume is to describe in detail category formation and read-across.

1.1.1 *In Silico* Predictions of Toxicity – Grouping, Category Formation and Read-Across

Similar objects tend to have similar properties. Applied to chemistry, this means that for chemicals that can be classed as being similar to other chemicals, we can understand and predict their properties without the need for testing. This fundamental concept has been applied to the prediction of properties and harmful effects of compounds for decades. Thus, being able to form groups of similar compounds (also called categories) becomes a powerful approach. If a compound belongs to a group of compounds with a well categorised toxicological profile, it can be possible to interpolate its activity. These interpolations, (predictions) of toxicity may, when utilised properly, provide hazard information that can be used in the assessment procedure described above. The process of prediction is termed "read-across" as it assumes that activities, toxicities or properties can be read across between compounds within a category.

Two hypothetical examples of read-across are provided in Figures 1.1 and 1.2 — these use data obtained from the OECD QSAR Toolbox version 3.1 (see Section 4.3 for more details). In the first example, Figure 1.1, a read-across prediction of *Salmonella typhimurium* gene mutation is made for 2-(3-ethylphenyl)oxirane. No mutagenicity data are available for this chemical. However, *S. typhimurium* gene mutation data are available for four closely related chemicals — termed analogues 1–4. These chemicals are considered "similar" as they all contain an aromatic ring, an epoxy group and limited alkyl substitution. The epoxy group allows the chemical to act as a direct acting electrophile by the S_N2 mechanism.[3] All the analogues to the target chemical are positive in the *S. typhimurium* gene mutation assay, they share the same structural features to the target, hence the read-across prediction for the target is that it will also share the same mechanism and be positive in this assay. This is therefore an example of a "qualitative" read-across.

The second hypothetical example is shown in Figure 1.2. This is a quantitative read-across in that a prediction is made for the acute fish toxicity of 3,4-dimethyl-1-pentanol. 96 hour LC_{50} values to the fathead minnow (*Pimephales promelas*) were retrieved for six analogues. These analogues are similar in that they are all simple saturated aliphatic molecules with a hydroxy group. As such they are assumed to act by the same mechanism of action — termed non-polar narcosis — and a good relationship is expected with the logarithm of the octanol-water partition coefficient (log P).[4] Figure 1.2 actually demonstrates the development of a local Quantitative Structure-Activity Relationship (QSAR), the line of best fit between toxicity and log P has the following equation:

$$\text{Toxicity} = 0.963 \log \text{P} + 0.769 \quad (1)$$

Where:

Toxicity is the inverse logarithm of the 96 hour LC_{50} values to *Pimephales promelas* (millimoles per litre).