

Chapter 3: The Scientific Basis of Chemical Risk Assessment

The Conventional Risk-assessment Paradigm

The risk assessment of chemicals has conventionally been based on four elements:

1. Hazard identification: the identification of the inherent capacity of a chemical to cause one or more adverse effects, without regard to the likelihood or severity of such effects.
2. Hazard characterisation: the (semi-)quantitative evaluation of the nature of adverse effects following the exposure to a chemical, including the assessment of toxic potency (the relative toxicity of a chemical) and, where possible, a dose–response assessment.
3. Exposure assessment: the (semi-)quantitative evaluation of the likely exposure of man and/or the environment to a chemical.
4. Risk characterisation: the (semi-)quantitative estimation of the probability that an adverse effect will occur, and of its severity and duration in a given population under defined exposure conditions, based on elements 1–3.

Shortcomings of Conventional Risk Assessment Approaches

The various elements of the risk-assessment paradigm encompass a variety of experimental activities. Hazard identification and characterisation (sometimes referred to collectively as hazard assessment) often rely on the use of animal experiments, whereas exposure assessment is generally the result of chemical analyses, but might also depend on bio-monitoring in animals or humans, and on computer-based estimations of exposure levels.

Animal-based studies generally lead to observations on the clinical, histopathological and/or functional changes in the animals caused by a given dose of the chemical under study. A common procedure is to determine the no observed adverse effect level (NOAEL) or the lowest observed effects level (LOEL). The NOAEL is the highest dose that produces no adverse effect in the most sensitive animal species, whereas the LOEL is the lowest dose that causes an adverse effect. An *adverse effect* can be defined as a change in the morphology, physiology, growth, development or life-span of an organism

that results in an impairment of its functional capacity or ability to compensate for additional stress, or in an increased susceptibility to the harmful effects of other environmental influences (1). *Relevance* depends on the risk evaluation to be made: if the aim is to evaluate the risk of an adverse effect in humans, the effect should be relevant to humans. Thus, an important question is whether the outcome of a particular animal experiment is relevant for the exposure of humans under practical circumstances.

Besides ethical objections against, and legal restrictions on, the use of animals for toxicity testing, there are also scientific reasons for reducing the current reliance on animal data. The specific shortcomings of various animal tests have been discussed elsewhere (2). More-generally, there are difficulties associated with the need to extrapolate: a) across species, from relatively small, but homogeneous, groups of laboratory animals to the very large and heterogeneous general human population; and b) from the high doses used to elicit effects in experimental animals to low doses, which are more consistent with human exposure levels. Thus, the use of animal data to predict the biological activities of compounds in humans is always prone to some degree of uncertainty. When assessing risk, toxicologists have conventionally attempted to overcome these uncertainties by introducing safety factors. For example, NOAELs determined from animal experiments are often divided by safety (i.e. uncertainty) factors to account for interspecies and/or inter-individual differences, when establishing safety standards for human exposure, such as an Acceptable Daily Intake (ADI; 3). Since it is considered acceptable to apply uncertainty factors when using animal data for risk assessment, it should also be acceptable to apply the concept of uncertainty when using *in vitro* data; for example, when extrapolating from cell culture systems to whole animals or humans.

Advances in the Scientific and Technological Basis for Risk Assessment

Over the last two decades, increasing emphasis has been placed on the development of non-animal test systems that are based on a fundamental biological understanding of the critical steps or events that link exposure to the expression of an adverse effect. The term *mode of action* is used to refer to one or more critical steps in the sequence of events

that leads to a toxicological response, whereas the term *mechanism of action* refers to the complete cascade of events (US EPA; 4). In general, modes of action are better understood than mechanisms of action.

Although the need for mechanistic tests is now widely recognised, it is not always clear what is meant by this term. For example, it could describe tests that involve biological systems with a mechanistic basis that is understood, or tests that are able to identify effects that are mechanistically related to the *in vivo* effects to be predicted. Frazier (5) defined a *mechanism* as an explanation of an observed phenomenon that explains the processes underlying the phenomenon in terms of events at lower levels of organisation. Thus, a *mechanistic test* is a test based on a system at an acceptable level of organisation and a relevant endpoint based on a sufficient understanding of the cellular and/or molecular basis of the effect under consideration. An example would be a test based on interaction with a specific receptor, which is known to be a critical step in the development of a toxicological effect.

Scientific and technological advances can be recognised in a few key areas. Developments in molecular biology and cell culture techniques, in physiological measurements, and in genomics and proteomics are contributing significantly to our understanding of the basic biochemical and physiological processes underlying toxicological responses, and therefore to the scientific and technological basis for toxicity testing. In addition, developments in the use of computer-based modelling techniques are providing increasingly powerful ways of using and integrating non-animal data for predictive purposes.

Novel *in vitro* methods, such as those based on genetically engineered cell lines, are being developed for the assessment of mechanistically relevant endpoints. For example, the use of genetically engineered mammalian cell lines with stable expression of specific human cytochrome P450 isoforms permits the study of metabolic activation, inhibition and the effects of human polymorphism (see the section on xenobiotic metabolism in Chapter 7). Such cell lines have also been used for the assessment of specific target-organ and target-system toxicities. Examples include novel systems for evaluating neurotoxicological hazard (6) and embryotoxic hazard (7).

Genomics and proteomics are areas of rapidly expanding knowledge. The application of genomics to toxicology is based on the assumption that most, if not all, toxic substances alter gene expression, so it should be possible to detect the potential toxicity of a chemical by screening for its ability to alter the expression of a diagnostic set of genes. In a study by Thomas *et al.* (8), DNA microarray analysis was used to determine changes in the levels of gene transcripts expressed in the livers of mice exposed

to five types of toxic chemicals: peroxisome proliferators, aryl hydrocarbon receptor agonists, non-coplanar polychlorinated biphenyls, inflammatory agents and hypoxia-inducing agents. Statistical analysis of the gene patterns identified a set of 12 diagnostic transcripts, out of a total of 1200 genes, which enabled the toxic chemical category to be predicted with 100% predictive accuracy.

Another area of increasing knowledge concerns the relationship between the structures/properties of chemicals and their toxicities. For example, the association of specific molecular fragments with toxicological endpoints has led to the development of structure–activity relationships (SARs), whereas physicochemical properties, such as lipophilicity, hydrophilicity and molecular mass, have been used in the development of quantitative structure–activity relationships (QSARs). Knowledge of the physicochemical characteristics of a chemical is also important for an understanding of its biokinetic behaviour. For example, quantitative structure–property relationships (QSPRs) have been used to predict biokinetic behaviour (9–11).

A fourth area, commonly referred to as “*in silico* toxicology”, has been the development of computer-based modelling techniques. Over the last fifteen years, the feasibility of computer-based approaches for predicting biokinetics has been greatly increased, because of the availability of computer techniques that permit the simultaneous, numerical solution of differential equations. In addition, there has been substantial progress in the development of computational techniques that can be used for QSAR and toxicodynamic modelling.

As a result of these scientific and technological advances, new toxicity tests can now be developed on the basis of an improved mechanistic understanding of toxicological processes, and individual tests of different kinds can be combined in the form of integrated testing strategies (12).

Approaches for Reducing the Amount of Testing

When the REACH system is implemented, several approaches could be adopted for reducing the extent of animal and/or non-animal testing necessary to meet a given set of information requirements. In order to derogate from a standard set of assessment requirements, the use of *read-across* is already accepted by some regulatory authorities, and the concept of *reverse risk assessment* also deserves serious consideration.

Read-across

It can be assumed that chemicals with similar physicochemical property profiles will generally have

similar toxicity profiles. Therefore, the clustering of chemicals with similar physicochemical property profiles into groups will permit *read-across* of toxicological properties within the groups, thus reducing the extent of testing required for chemicals within the group. Read-across is the process by which one or more toxicological properties of a given chemical are inferred by comparison of that chemical with chemicals of similar molecular structures and physicochemical properties, for which the toxicological properties of interest are known. The process involves an appraisal by a regulatory authority, on the basis of information provided by industry.

A number of issues need to be considered when assessing the toxicological properties of a new substance by read-across: a) the similarity of the purity and impurity profiles of the new substance and the structural analogue needs to be assessed, since there should be no differences in the purities or impurities on a scale that would be likely to influence the overall toxicity; b) the physicochemical properties of the new substance should be compared with its analogue (in particular, the physical form, molecular mass, water solubility, partition coefficient and vapour pressure provide useful information as to similarity); c) the likely toxicokinetics of the substances, including the possibility of different metabolic pathways, should be considered; d) if read-across data on structural analogues have not been produced by using current Annex V test methods or current OECD test guidelines, caution should be exercised when extrapolating these data to a new chemical; and e) regulatory authorities are more likely to accept the read-across of positive findings (presence of toxicity), rather than negative findings (absence of toxicity).

As an illustration of the use of read-across in a regulatory submission for new chemicals, a request received by the UK Health and Safety Executive involved a series of four structurally similar substances differing in their numbers of carbon atoms. The result was full base-set testing of the lowest homologue of the series, and limited testing on the highest homologue. For the other group members, all the toxicological data used for base-set notification were read-across data (Rosalind Hanaway, personal communication).

Further information on the use of read-across in a regulatory context is given by Hanway & Evans (13).

Other hypothetical examples of read-across are provided by Barratt (14). For example, in a homologous series of surfactants (alkyl betaines) with C₈, C₁₀, C₁₂, C₁₄ and C₁₆ alkyl chains, the systemic toxicities of the C₁₀ and C₁₄ chemicals could be predicted from tests carried out on the C₈, C₁₂ and C₁₆ members of the series. This would result in a 40% reduction in animal usage.

Reverse risk assessment

A *reverse risk assessment* enables the extent of testing needed for a chemical to be judged according to its prospective use and exposure conditions, instead of the performance of a standard list of hazard-assessment tests followed by a risk assessment. In the latter situation, the margin of safety eventually obtained could be so high that it would be realised that much of the testing was unnecessary in the first place. The margin of safety is the magnitude by which the NOAEL exceeds the known or estimated exposure level.

The first step is to perform an exposure assessment, based on experimental measurements and/or predictions. Once the likely exposure is known or predicted, a NOAEL (or LOEL) is derived by measuring organ-specific cytotoxicities. If the likely exposure is significantly less than the NOAEL (i.e. the chemical has a lower toxicity than would cause concern in the event of the likely exposure), then no more testing is required. Since the NOAEL is obtained by using isolated cells, this implies that 100% of the chemical is absorbed. If the NOAEL is of the same order or slightly less than the exposure level, then the possibility that absorption is less than 100% should be considered when deciding whether further testing is necessary. Conversely, if the NOAEL is significantly less than the exposure level, it may be necessary to undertake additional testing.

A proposal for an exposure-driven risk-assessment process has been developed by CONCAWE, the European oil-industry organisation for environment, health and safety (unpublished document submitted to the European Commission Working Group on Testing, Registration and Evaluation). According to this proposal, the extent of testing considered necessary is determined by using an objective measure of exposure, based on tonnage, use category, and physicochemical properties.

The Use of Alternative Methods in Hazard and Risk Assessment

The use of physicochemical data by QSAR

Physicochemical data are useful at an early stage in the hazard/risk assessment process, since it may be possible to extrapolate them to toxicological effects by means of QSAR models (in which the descriptor variables are physicochemical properties). In the context of the future chemicals policy, QSARs based on the physicochemical properties required for the base-set notification would be particularly useful. These properties are summarised in Table 3.1.

The use of *in vitro* data

The use of *in vitro* tests in different parts of the risk-assessment process (hazard identification, hazard characterisation and risk assessment) was discussed by Balls & Fentem (15). As described in Chapter 2, the data generated by *in vitro* tests can be extrapolated to toxicological effects by using prediction models (PMs). In principle, various PMs can be applied to the data generated by a particular *in vitro* test. Classification models can be used to identify chemicals that are potentially hazardous (hazard identification), and to predict their regulatory classifications (hazard classification). Examples include PMs for predicting skin corrosion potential (16), such as the following PM, based on pH measurements (OECD, 17):

*if the pH of a substance ≤ 2 , or if $\text{pH} \geq 11.5$,
classify as a corrosive.*

If different classification systems exist for a particular type of toxic hazard (for example, the EU system and the Globally Harmonised System [GHS]), different PMs might need to be applied to the same *in vitro* data. For the purposes of risk management, the classification of chemicals might be considered to be a sufficient basis for making certain decisions, such as on fast-track risk-reduction measures.

In other cases, it might be necessary to perform a more-comprehensive hazard characterisation; for example, to obtain an estimate of toxic potency. In

Table 3.1: Physicochemical properties required for the base-set notification

No.	Physicochemical property
1	Melting point (melting range) ^a
2	Boiling point (boiling range) ^a
3	Relative density
4	Vapour pressure ^a
5	Surface tension
6	Water solubility
7	Fat solubility
8	Partition coefficient (log P)
9	Flash point ^a
10	Flammability
11	Explosive properties
12	Auto-flammability ^a
13	Oxidising properties
14	Abiotic degradation: hydrolysis as a function of pH (a test requirement for ecotoxicity)

^aThe physical state of the chemical may make the determination of some of these properties inappropriate.

such cases, a different kind of PM is required, which could be a regression model. An example is a PM for estimating acute lethal doses (LD50 values) from cytotoxicity (IC50) data (18).

If a risk assessment is required, *in vitro* dose-response data would need to be used in combination with exposure measurements or estimates, taking into account biokinetic considerations (see Chapter 7 for further details).

The integrated use of physicochemical data and *in vitro* data

Finally, it is recommended that the assessment of toxicological endpoints is based on the intelligent and combined use of physicochemical and *in vitro* data, in the form of tiered strategies. Examples include the tiered testing strategies for acute dermal and ocular toxicity, described in Chapter 5. The development and validation of such strategies should be a matter of high priority, since they combine the strengths of complementary approaches.

References

1. IPCS (1978). *Principles and Methods for Evaluating the Toxicity of Chemicals. Part I. International Programme on Chemical Safety. Environmental Health Criteria 6*. (Accessed 13.6.02) pp. Geneva, Switzerland: WHO. Web site: http://www.who.int/pes/pubs/pub_meth.htm.
2. BUAV (2001). *Action to End Animal Testing. The Way Forward*, 19 pp. London, UK: British Union for the Abolition of Vivisection.
3. Kroes, R. & Feron, V.J. (1990). Toxicity testing: strategies and conduct. In *Progress in Predictive Toxicology* (ed. D.B. Clayson, I.C. Munro, P. Shubik & J.A. Swenberg), pp 15–39. New York, NY, USA: Elsevier Science.
4. EPA (1999). *Guidelines for Carcinogen Risk Assessment*. Risk Assessment Forum. NCEA-F-0644. Washington, DC, USA: Environmental Protection Agency.
5. Frazier, J.M. (1994). The role of mechanistic toxicology in test method validation. *Toxicology in Vitro* **8**, 787–791.
6. Stingele, S., Coecke, S., Nicotera, P. & Balls, M. (1999). EC Patent filed 27th August 1999 on *Genetically Engineered Cell Lines, and Their Uses, in Particular for Neurotoxicity Testing*. Patent No. 99402139.2–2106/International No. PCT/EP00/08223. Rijswijk, The Netherlands: European Patent Office.
7. Bremer, S., Worth, A.P., Paparella, M., Bigot, K., Kolossov, E., Fleischmann, B.K., Hescheler, J. & Balls, M. (2001). Establishment of an *in vitro* reporter gene assay for developmental cardiac toxicity. *Toxicology in Vitro* **15**, 215–223.
8. Thomas, R.S., Rank, D.R., Penn, S.G., Zastrow, G.M., Hayes, K.R., Pande, K., Glover, E., Silander, T., Craven, M.W., Reddy, J.K., Jovanovich, S.B. & Bradfield, C.A. (2001). Identification of toxicologically predictive gene sets using cDNA microarrays.

- Molecular Pharmacology* **60**, 1189–1194.
9. DeJongh, J., Verhaar, H.J. & Hermens, J.L. (1997). A quantitative property–property relationship (QPPR) approach to estimate *in vitro* tissue–blood partition coefficients of organic chemicals in rats and humans. *Archives of Toxicology* **72**, 17–25.
 10. DeJongh, J., Verhaar, H.J.M. & Hermens, J.L.M. (1998). Role of kinetics in acute lethality of nonreactive volatile organic compounds (VOCs). *Toxicological Science* **45**, 26–32.
 11. Soffers, A.E.M.F., Boersma, M.G., Vaes, W.H.J., Vervoort, J., Tyrakowska, B., Hermens, J.L.M. & Rietjens, I.M.C.M. (2001). Computer-modeling-based QSARs for analyzing experimental data on biotransformation and toxicity. *Toxicology in Vitro* **15**, 539–551.
 12. Blaauboer, B.J., Barratt, M.D. & Houston, J.B. (1999). The integrated use of alternative methods in toxicological risk evaluation. ECVAM integrated testing strategies task force report 1. *ATLA* **27**, 229–237.
 13. Hanway, R.H. & Evans, P.F. (2000). Read-across of toxicological data in the notification of new chemicals. *Toxicology Letters* **116**, Suppl. 1, 61.
 14. Barratt, M.D. (2000). Principles of toxicity prediction from chemical structure. In *Progress in Reduction, Refinement and Replacement in Animal Experimentation* (ed. M. Balls, A-M. van Zeller & M.E. Halder), pp. 449–456. Amsterdam, The Netherlands: Elsevier.
 15. Balls, M. & Fentem, J.H. (1992). The use of basal cytotoxicity and target organ toxicity tests in hazard identification and risk assessment. *ATLA* **20**, 368–388.
 16. Fentem, J.H., Archer, G.E.B., Balls, M., Botham, P.A., Curren, R.D., Earl, L.K., Esdaile, D.J., Holzhütter, H.G. & Liebsch, M. (1998). The ECVAM international validation study on *in vitro* tests for skin corrosivity. 2. Results and evaluation by the Management Team. *Toxicology in Vitro* **12**, 483–524.
 17. OECD (1998). *Harmonized Integrated Hazard Classification System for Human Health and Environmental Effects of Chemical Substances*, 65pp. Paris, France: Organisation for Economic Cooperation and Development.
 18. Spielmann, H., Genschow, E., Liebsch, M. & Halle, W. (1999). Determination of the starting dose for acute oral toxicity (LD50) testing in the Up-and-Down Procedure (UDP) from cytotoxicity data. *ATLA* **27**, 957–966.