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Toxicology: a Science and an Art

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Abstract—Toxicology is both a science and an art. The science of toxicology characterizes the adverse effects of a chemical by integrating all available scientific information. Toxicologists must ask whether current protocols truly characterize the toxicity of a chemical in terms of identifying all the potential adverse effects. The art of toxicology involves the use of a database to predict toxic risk. A key question for the future of toxicology is "can alternative models contribute to progress or are they useless?" This paper concludes in favour of progress if such alternative models help to improve the science of toxicology. As a practical approach to improve the art of toxicology it proposes reinforcement of flexibility of current regulations, encouragement of the production and submission of data of alternative tests, and evaluation of these data on a strictly scientific basis. ECVAM, the European Centre for the Validation of Alternative Methods, will play a major role in implementing this new and more scientific approach to toxicology.

What is Toxicology?

Toxicology is the **science** that studies the properties of chemicals and their effects on living organisms with the aim of assessing (the **art**) their potential adverse effects, in order to help preserve and protect human health.

The **science** of toxicology characterizes the adverse effects (the biological reactivity) of a chemical by integrating all available scientific information. This includes the following: the nature and chemical properties of the chemical; the adverse effects in relevant biological systems; the dose–response relationship; the mechanism of action; the influence of exposure conditions; the species, strain, age and sex differences. However, at present, the scientific toxicity database consists mainly of the results of studies carried out in two or three species of animals. These studies are termed 'safety evaluation tests' with the questionable implication that a chemical can be judged safe for humans if it produces negative results in a series of 'scientifically' conducted protocols, most exclusively in experimental animals.

Toxicologists should query whether these protocols truly characterize the toxicity of a chemical in terms of identifying all the potential adverse effects for humans and domestic animals and establishing the relevant database. Furthermore, they should ask whether and, most importantly, how the science can be improved (Kroes and Hicks, 1990). In that respect it is disquieting to realize that toxicologists have still only a limited understanding of the mechanisms through which most chemicals exert their toxic effects and, as yet, have only a limited capacity to predict the adverse effects of a given chemical in an intact animal, particularly in humans. However, in the rush to regulate, these limitations are frequently forgotten and the use of what appear to be precise estimates of risk belies the uncertainties that exist and often vastly exaggerates the capacity of the 'Science of Toxicology' (Wilkinson, 1986).

The **art** of toxicology involves the utilization of a (scientific) database to predict toxic risk, defined as the probability that a particular adverse event occurs during a stated period of time or results from a particular challenge (Warner, 1986). In the case of a chemical, it is a function of the intrinsic capacity of the material to cause an adverse effect and the dose—usually determined by the intensity, frequency and duration of exposure. Being predicted, the risk must still be assessed, first by risk estimation and secondly by risk evaluation, both leading to risk management—the process of decision-making concerning risks and their subsequent implementation. It is the process whereby an appropriate regulatory decision is reached that will obviate, minimize or otherwise manage the risk. Risk management is not *per se* a scientific process; it involves value judgements through which the regulator attempts to balance the risks against a variety of other factors (benefits, costs, political aspects, etc.) that depend on the statute under which regulatory action is being considered (Wilkinson, 1986).

Owing mostly to the generally poor understanding of the mechanisms of toxicity, the risk assessment process relies too much on theoretical models (for example, the two-step hypothesis for carcinogenesis). Furthermore, it is too dependent on controversial and unsubstantiated assumptions (for example, the pertinence of a linear dose–effect relationship to support the extrapolation of extremely high-dose effects to low-dose effects). All too often, when considering a model, statistical arguments overrule

Abbreviation: ECVAM = European Centre for the Validation of Alternative Methods.

scientific logic and the **science** and **art** of toxicology come second (Wilkinson, 1986). This procedure fails to give further consideration to a possible extensive additional database composed of scientific information resulting from alternative tests, and which is simply ignored. Currently used experimental models for collecting data do not, of course, yield the best evaluation about human risk, and it is the lack of fundamental biological knowledge that is the major bar to this evaluation.

The question thus becomes one of whether toxicologists are using the correct models (Rodricks, 1986) and what they can do to improve these models. With regard to the so-called alternative models, the question must be asked: can they contribute to progress in toxicology or are they useless?

What is the future of the science in toxicology?

Essential components of advances in toxicology are the investigation of mechanisms of toxicity and the development of assay systems for specific types of biological injury by using experimental techniques from many disciplines. The toxicologists that will be needed for the future are those scientists who, by their research and experience, are able to link the many disciplines involved into coherent concepts of toxicity.

To meet such guidelines, as recommended by the Society of Toxicology's TOX-90's commission (Miya *et al.*, 1988): 'new technology and knowledge developed by all relevant disciplines must be integrated into toxicology; progress in toxicology demands that the discipline must increasingly address good science and the scientific methods'.

Without any doubt, the second half of the 20th century will remain in history as the era of the explosion of knowledge in biological science. Highly sophisticated techniques have been developed that have allowed immense steps forward in the understanding of biology. Yet toxicology, as a science, ignores most of the mechanisms of the effects it observes. Compared with biology, as well as with its sister discipline, pharmacology, toxicology as a science is still in its infancy. The future of toxicology requires a rapid and immense effort to integrate these techniques with the objective of performing more basic research. This is the only way to develop better and more predictive tests, the rationale of which will be the identification of the cellular and molecular mechanisms of the adverse effects. Continuing advances in toxicology will depend largely on how well scientists in this discipline are able to transfer and integrate cutting-edge technology from all the relevant sciences and exploit them to the full in solving problems in toxicology (Miya *et al.*, 1988). As stated by the US National Academy of Sciences (NAS) in a report on *Risk Assessment in the Federal Government: Managing the Process*: "the basic problem ... is the sparseness and uncertainty of the scientific knowledge of the health hazards addressed" (NAS, 1978). To improve the science of toxicology it is necessary to create a mechanism for research on the scientific issues underlying risk assessment. The stated and actual purpose of such a mechanism should be the very best science, and not to test products or to assess risks (Press, 1984).

Alternative and complementary approaches in toxicology must be developed, based on a far greater understanding of the interaction between chemicals and biological systems. To do so, the science of toxicology must become increasingly oriented towards the use and application of all the models, methodologies and techniques available today.

Both scientific arguments (explosion of basic knowledge in biology, development and improvement of bioanalytical techniques, progress in cell biology) and ethical considerations plead for the development of *in vitro* methods as the major clue to improve the science of toxicology, even if this must be a long-term goal. *In vitro* systems (tissue slices, primary cell culture, cell lines, subcellular fractions) are indeed obligatory means for basic research on the mechanisms of toxicity; they offer the possibility of using human materials, and they can easily be organized as a flexible screening battery to cover a wide

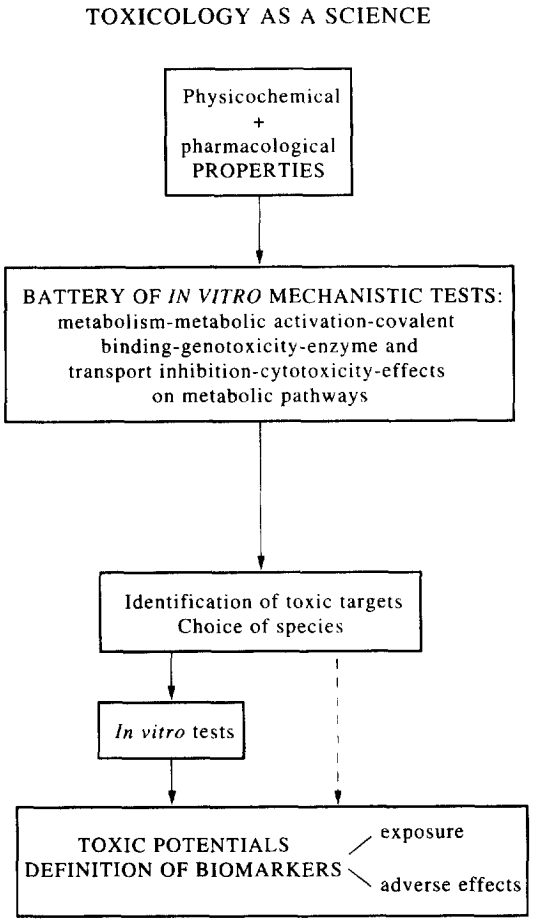

Fig. 1. Information necessary for characterization of the toxic potential of a chemical.

range of mechanistically described adverse effects. Figure 1 lists the most important information needed to characterize the toxic potential of a chemical. Tests are already available (or can easily be made available) to generate such information. The advantages of such an approach are an extensive knowledge of molecular and cellular events related to toxic potential, the opportunity of choosing adequate species for *in vivo* tests if required, the possibility of defining selected targets for such tests, and the identification of biomarkers of exposure and adverse effects.

On the basis of such information, it will then be possible to draw scientific conclusions with regard to the potential adverse effects of the chemical that could be related to its toxic potential. Such conclusions might then be used to design further tests, including *in vivo* animal experiments, with the aim of obtaining the scientifically based information necessary for risk assessment.

New tests in toxicology will have to be scientifically valid. They will have to observe and describe scientifically the adverse effects on the basis of precise knowledge of their mechanisms. With regard to the science of toxicology, that is the only validation required. *In vitro* toxicology as a science will find its validation in its own practice, its only objective being to provide toxicologists with the best available scientific information.

What is the future of the art in toxicology?

If, as discussed above, toxicology becomes more scientific, what will then be the future of the art of toxicology? On the basis of a more scientific database, the prediction of risk is anticipated to be of higher value and the probability that a particular adverse effect occurs in humans will be better defined. As required by the *ad hoc* committee on TOX-90 (Miya *et al.*, 1988) "the art of toxicology will be improved because it will base prediction on a thorough understanding of how and why the data (are) generated, what they mean and how they (can be) employed. Better science will lead to a better art, but the art has to adapt".

With regard to the procedures for toxicity testing, the rigid approach that requires that all compounds should be subjected to the same comprehensive battery of test procedures using large numbers of animals must be abandoned in favour of a more flexible approach that requires only those data necessary to provide an evaluation of risk of the particular compound tested (Kroes and Feron, 1984). This approach obviously places a greater responsibility on the toxicologists who design and conduct tests and who describe observations and draw conclusions.

The art of toxicology must, in future, abandon an approach that, owing to the limited scientific basis of current practice, has developed more as a regulatory than a scientific approach. The development of new tests (mostly *in vitro*) and the improved knowledge of the mechanisms of the adverse effects will provide new information that cannot be ignored but that requires evaluation to serve in risk assessment. One of the possible schemes for toxicity testing in the development of a new chemical could be extensive screening for biological (cellular, biochemical) reactivity based exclusively on a battery of *in vitro* test systems. Such a battery could cover the major types of cells and tissues and it should detect effects on the major biochemical and physiological functions, as well as identifying the major metabolic pathways of the chemicals. It should, as often as possible, favour the use of human material. The integrated analysis of such scientific information (Fig. 2) should enable the toxicologist better to design further experiments, including *in vivo* tests in animals possibly followed by early tests in human volunteers. At the conclusion of such a scientifically based trial, the toxicologist must be in a position to evaluate the risk, participate in the design of molecular modification of the tested compound and advise the regulator. Finally, by reference to the scientific data available, the toxicologist will be in a position to identify potential toxic effects and thus to design a limited number of scientifically justified *in vivo* experiments, using the most relevant species (as compared with humans). *In vitro* toxicology, as part of the art to predict the probability to find a given adverse effect in humans, will have to find its validation, which cannot be merely a comparison with current practice (Roberfroid, 1991). Such a validation must take place as a result of consideration of a specific purpose for which the test is developed.

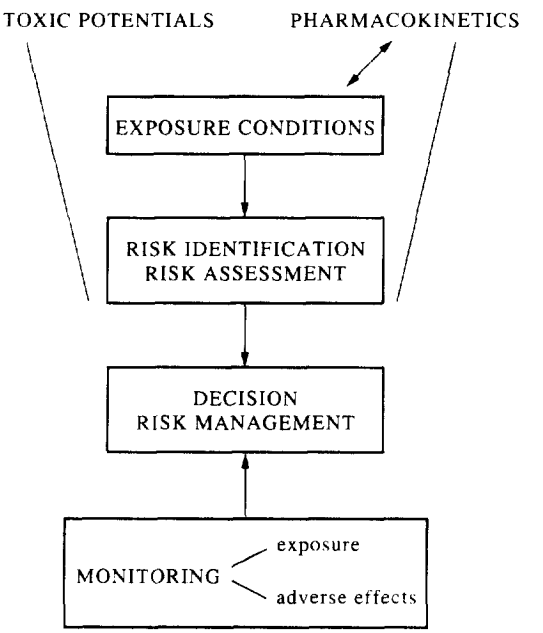

Fig. 2. Integrated analysis of scientific data from toxicity testing in the development of a new chemical.

Risk assessment of chemicals: how to improve current practice?

In the process of risk assessment of chemicals, toxicologists have to provide information for three major but distinct purposes:

1. Classification of chemicals as required, worldwide, for handling and transport;
2. Identification of 'gross mostly local toxic effects' with the priority aim of protecting workers and professional users of reactive chemicals from, for example, eye and skin irritation;
3. Assessment of the risk to the general population being exposed, deliberately or accidentally, to food additives, food and environmental contaminants, drugs, cosmetics, etc.

To determine whether alternative tests will constitute an advance or will be useless for the risk assessment of chemicals, each of these purposes has to be discussed separately.

Classification of chemicals

Classification of chemicals has traditionally been based on LD_{50} tests. Thanks to a large international interlaboratory validation co-ordinated by van den Heuvel *et al.* (1990) from the UK and supported financially by the EEC, such a classification is now based on the refined procedure known as the fixed-dose procedure (FDP), which initially was developed in the UK under the auspices of the British Toxicology Society. Such an exercise did not require much scientific development; the validation procedure was accepted by all participants; it took place and the data demonstrated that, on the basis of the new test, chemicals would be classified in a similar manner to that based on the old test. Decision-makers at the EU, the Environmental Protection Agency (EPA) and the Organisation for Economic Cooperation and Development (OECD) have been informed by the scientists, and suitable action has been taken to adapt the legislation. This is an example of what Balls (1992) characterized as a "political validation".

Even if, in that particular example, no dramatic progress has been made in the science of toxicology, the ethics have improved and thus definite progress has been made. The next question is therefore is it unrealistic to propose the same exercise to be repeated with the aim of validating an *'in vitro* approach', based on which an acceptable classification of chemicals could be achieved without any use of animals? Once again, that exercise would not require extensive scientific development but rather an inventive empirical approach offering a limited battery of tests that could perform the task. It is my personal opinion that this is perfectly possible.

Gross, mostly local, toxic effects

Gross, mostly local, toxicity is classically evaluated on the basis of the results of highly controversial skin and eye irritation tests. Alternatives have been developed and have been (or are currently) included in large validation studies worldwide. However, it seems that this validation process is endless, the major arguments being that (a) the proposed alternatives have a weak scientific basis because they do not rely on understanding of the mechanisms of irritation, and (b) the validation is 'retrospective' and it cannot be excluded that, in the future, if the new approach is adopted, false negative results would appear.

If, once again, the results of such tests have to be used to classify the chemicals as strong, medium or mild irritants, then a battery of tests, for which one could substitute, or to which could be added, a more 'mechanistically' based test, could reasonably be accepted for a 'political-type validation'. There is no need for long-term, expensive and time-consuming scientific research. New tests can simply be validated, based on comparison to achieve a strictly limited but essential objective—to grade irritancy and, consequently, to improve protection.

Nevertheless, the argument of a risk of false negative results has to be taken into consideration. Even if the animal tests guarantee a 'safe evaluation of the safety' no more than any other, they have still, by virtue of their extensive use, gained a status of confidence that is difficult to ignore. A practical solution to that problem would be to require, for a given period, the results of both the classical and the alternative approaches to be submitted at the same time. By a continuing comparison of the data, scientists and regulators would be able, in a progressive process of evaluation, to come to a final decision.

Assessment of risk to the general population

Risk assessment for drugs, cosmetics, food additives and contaminants is, without doubt, the most difficult task for toxicologists because it concerns systemic toxicity. It implies identifying and characterizing the effects on all tissues, organs and functions. Moreover, it has to take into account metabolism and pharmacokinetics.

It appears, today, to be relatively unsound to anticipate that the results of a battery of *in vitro* tests will positively and strongly correlate with those of *in vivo* tests. Successful validation of a battery of *in vitro* tests is thus very unlikely, if not hopeless. *In vitro* tests alone cannot be used to predict systemic toxicity. However, toxicology, as a science, has made extensive progress and extremely powerful *in vitro*/alternative tests are available that provide useful information likely to improve risk assessment, either as adjunct, complementary or simply new tests. Since the final goal of toxicology is to improve safety evaluation/risk assessment, it has become evident that such information simply cannot be ignored merely because *in vivo* tests are the only tests accepted

or required. How can the following paradox be solved?

Alternative tests do exist that are likely to improve the quality of risk assessment.

No such test or battery of tests is likely to be validated to replace animal tests.

Everybody will agree that toxicology can no longer merely ignore the extremely valuable information these tests are likely to generate.

How can we solve that problem by making these tests contribute to progress in risk assessment? I do not see any other solution than the following:

1. Reinforcement of the flexibility of present regulations to permit and even stimulate a more scientific approach to risk assessment. The present rigidity that imposes a fixed set of tests must definitely be abandoned.
2. Encouragement (or even stipulation) of non-animal/alternative tests, with incorporation of their results, together with those of scientifically justified classical animal tests, in the dossier as it is submitted to the regulatory authorities.
3. Requirement that these authorities evaluate the results of both the animal/classical and the non-animal/alternative tests for risk evaluation on a strictly scientific basis.

We all know that these alternative tests are already in use both in industry and academia, mostly but not exclusively for screening. If we want the future practice of risk assessment both to be improved and to rely less on animal tests, my opinion is that we need an interim period during which the results of non-animal/alternative tests will be required and used in risk evaluation together with the results of classical/animal tests. Such a practice will enable us, on a practical basis, progressively

to convince the regulators of the quality and the value of alternative tests;

to elaborate valuable new tests for risk assessment;

to correlate *in vitro* with *in vivo* data;

to validate such alternative tests, in due course, not by comparison but on the basis of their intrinsic value.

In other words, a long-term exercise of collaboration between basic research, screening, mechanistic studies, and *in vitro* and *in vivo* testing is a valuable and feasible proposal if we want alternative non-animal tests to contribute to progress in risk assessment.

Rather than continuing the relatively useless empirical validation of *in vitro* versus *in vivo* tests, the above proposal could initiate a process of scientific validation, the objective of which would be, in time, to improve the science of toxicology, which we have initially defined as the major challenge that we have to face.

Conclusion

In conclusion, I wish to endorse the remarks made by F. Press when introducing a symposium on 'Safety Assessment, the Interface between Science, Law and Regulation', sponsored by ILSI in the USA in 1983: "Future decisions on risk will, I have no doubt, share some of the illogical history of many of today's practices, but what I would bet on is that the problems of risk will become less burdensome, your prospects more predictable, and acute risk better defined as we improve the science of toxicology. (But) for that to happen, we all have to act!" (Press, 1984).

My answer to the question of whether alternative tests in risk assessment constitute progress or are useless is thus clearly in favour of progress, since they will improve the science of toxicology. However, such progress requires inventive and perhaps revolutionary solutions, for which I have made a proposal.

ECVAM, the European Centre for the Validation of Alternative Methods, can and must play a major role in favour of this progress. Its roles are definitively to strengthen the science of toxicology by calling on experts throughout Europe to identify the most relevant molecular and cellular events that play a role in the toxicity of chemicals and to design research programmes aimed at developing relevant tests to identify/quantify such events. Subsequently, ECVAM will have to co-ordinate such research programmes with the objective of validating/codifying such tests and ultimately of building up a battery of mechanistic tests. In parallel, it will have to develop the concepts necessary to define toxic potential on the basis of such scientific information and to train toxicologists in such new approaches.

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