Chapter 11

Toxicity Testing and Risk Assessment

Introduction

In most industrialized countries, drugs (including veterinary medicines), food additives and contaminants, industrial chemicals, pesticides and cosmetics, to which humans and other living organisms in the environment may be exposed, have to be tested for toxicity. The regulations can vary between countries, however, and it is not within the scope of this book to discuss the regulations in any detail. More detail of the regulations may be gained from the references in the Bibliography. The purpose of Regulatory Toxicology is to ensure that the benefits of chemical substances intended for use by humans outweigh the risks from that use.

The conduct of the toxicity tests required depends partly on the type of substance and its expected use and also on the regulations of the particular country. The amount of data necessary also depends on the end use of the substance. For instance, industrial chemicals produced in small quantities may require only minimal toxicity data whereas drugs to be administered to humans require extensive toxicological testing. Pesticides may have to be tested for their effects on many different types of animal and plant in the environment and examined for their persistence.

Toxicity tests all share certain basic principles. They usually involve exposing experimental animals or plants to the test substance under controlled conditions. For existing chemicals, however, toxicological information may also be obtained from humans and animals such as those given drugs during clinical trials, individuals exposed in the work place and humans and other animals exposed in the general environment. Such epidemiological evidence can be extremely important.

Thus, the monitoring of exposure by measuring substances and their metabolites in body fluids and using biochemical indices of pathological change may be carried out in humans during potential exposure (see Detection of toxic responses, Chapter 3). An example is the monitoring of agricultural workers for exposure to organophosphorus compounds by measuring the degree of inhibition of cholinesterases in blood samples. Studying particular populations of predatory birds and measuring certain parameters, such as eggshell thickness and pesticide level, is an example of testing for toxicity in

the field. For human and veterinary medicines in the UK there is a system for reporting adverse reactions to drugs: for human medicines this is the yellow card system; for veterinary drugs adverse reactions of both the animal patient and the human user are reported. Data relating to exposure is vitally important in the eventual assessment of the whole toxicological database for a particular compound.

Pesticides and other chemical substances which can contaminate the environment will also need to be examined for their persistence in the environment and their behaviour in food chains. The stability of such compounds in particular environments is also of importance. Consequently, ecotoxicology involves more extensive residue analysis than does drug toxicology for example. The exceptions to this are veterinary medicines where the estimation of residues in animals intended for human consumption is vitally important.

Examples of pertinent questions which should be asked before any toxicity study are:

1. is it a novel compound or has it been in use for some time?;
2. is it to be released into the environment?;
3. is it to be added to human food?;
4. is it to be given as a single dose or repeatedly?;
5. at what dosage level is it to be administered?;
6. what age group will be exposed?;
7. are pregnant women or women of childbearing age likely to be exposed?.

Toxicity may be an intrinsic property of a molecule which results from interaction with a particular biological system. Consequently, a knowledge of the physico-chemical properties of that molecule may help the toxicologist to understand the toxicity or potential toxicity and to predict the likely disposition and metabolism. Indeed, we have seen several examples in this book of the importance of physico-chemical principles in toxicology. Structure-activity relationships are beginning to be used in toxicology as they are in pharmacology, especially in the field of chemical mutagenesis\carcinogenesis. This initial knowledge from preliminary studies may also influence the course of the subsequent toxicity tests especially if there are similarities with other compounds of known toxicity. Hence, the solubility, partition coefficient, melting or boiling point, vapour pressure and purity are important parameters. For example, an industrial chemical which is a very volatile liquid (i.e. with a high vapour pressure) should at least be tested for toxicity by inhalation and possibly by skin application.

As well as physico-chemical considerations there are also biological considerations and the following are the major ones:

1. the most appropriate species to study,
2. the sex of the animals used,
3. the use of inbred or outbred strains,
4. housing,
5. diet.

6. animal health,
7. metabolic similarity to man,
8. the route of administration,
9. duration of the toxicity study,
10. the numbers of animals used,
11. vehicle.

The route of administration and vehicle will depend on the expected end use or, if a drug for example, on the means of administration. The parameters to be measured may also be dependent on the particular study. For example, metabolic studies can be combined with a toxicity study and plasma levels measured as well as urinary metabolites identified and clinical chemical parameters studied. The biochemical and pathological measurements to be made will also be decided before the study is started.

Initial toxicity studies will usually be carried out to determine the approximate range of toxic dosage. For a drug this may already be known from pharmacological studies but for an industrial chemical, for instance, nothing may be known of its biological activity. Consequently, the initial range-finding studies may utilize dosage on a logarithmic scale or half-log scale. These initial studies are important if large numbers of animals are not to be wasted in later studies. The initial tests will also involve observation of the animals in order to gain insight into the possible toxic effects.

Once the approximate toxic dosage range is known then various detailed toxicity studies can be carried out. These will be followed by various other toxicity tests, usually including the following: acute, sub-acute (28- or 90-day), chronic (lifetime), mutagenicity, carcinogenicity, teratogenicity, reproductive studies and *in vitro* tests. For some compounds there may also be other types of toxicity test such as irritancy and skin sensitization studies.

There are different requirements for drugs, food additives and contaminants, industrial chemicals, cosmetics and pesticides because of the different circumstances of exposure. Chemicals which are to be used in the environment, such as pesticides and industrial chemicals which might be accidently released into the environment, will also undergo ecotoxicity tests. These will include tests with invertebrates such as *Daphnia,* earthworms, fish, phytoplankton and higher plants.

Acute Toxicity Tests

Acute toxicity tests are those designed to determine the effects which occur within a short period after dosing. These tests can determine a dose-response relationship and the LD_{50} value. The exact conduct of toxicity studies will vary depending on the compound, its eventual use and the particular regulations to be satisfied. Usually at least four dosages are used which may be in logarithmic progression especially if no range-finding studies have been done. Although the traditional LD_{50} determination is now less popular with many toxicologists it is still required by some regulatory authorities. (For more information on

Table 11.1. Investigation of acute oral toxicity and estimation of maximum non-lethal oral dosage for classification purposes.

Test dosage	Result	Action/classification
5 mg kg^{-1}	<90% survival	*Very toxic*
	>90% survival but toxicity	*Toxic*
	>90% survival no toxicity	Retest at 50 mg kg^{-1}
50 mg kg^{-1}	<90% survival	*Toxic*; test/retest at 5 mg kg^{-1}
	>90% survival but toxicity	*Harmful*
	>90% survival no toxicity	Retest at 500 mg kg^{-1}
500 mg kg^{-1}	<90% survival or toxicity	*Harmful*; test/retest at 50 mg kg^{-1}
	>90% survival no toxicity	*Unclassified*

This table has been adapted from M.J.van den Heuvel *et al.,* Human Toxicology, 6, 279, 1987.

this test see the publications in the Bibliography.) Recently an alternative to this test which attempts to find the approximate toxic dosage but uses far fewer animals has been suggested by the British Toxicology Society. In this procedure a small number of animals, such as five of each sex, are exposed to the chemical under test at a dosage level of 5 mg kg^{-1} (for example) and observed for signs of toxicity. If 90 per cent or more of the animals survive without signs of toxicity then a larger dosage, such as 50 mg kg^{-1} is employed. If again 90 per cent or more survive without signs of toxicity then the chemical is termed unclassified. Depending on the dosage required for toxicity to be evident then the chemical can be classified as shown in Table 11.1.

The information to be gained from an acute toxicity test is the nature of the dose-response relationship and observations on the toxic effects and time to death, if any of the animals die. The LD_{50} value may also be determined if sufficient animals at each dosage level have been used. It is important that the dosage range used is wide enough for toxic effects to be manifested at the highest dosages used unless this would require doses that were unrealistic in relation to the expected dose or exposure. The dosage range and the method of administration will be influenced by the expected or intended route of administration and likely dosage or exposure concentration.

At the end of the toxicity test the surviving animals are killed and undergo a post-mortem with a pathological examination of tissues. Animals dying during the study should also undergo a post-mortem.

Sub—Acute Toxicity Tests

Following acute toxicity tests, sub-acute toxicity tests are usually carried out. These involve exposing the animals to the substance under test for a prolonged period, usually 28 or 90 days. The exposure is frequent and usually daily. The sub-acute tests which are also known as sub-chronic tests, provide information on the target organs affected by the compound and the major toxic effects. Toxic effects which have a slow onset can be detected and reversible and adaptive responses may become apparent during the test. Measurements of

levels of the compound in blood and tissues can be made and this information correlated with any toxic effects seen. At the end of the study pathological examination is carried out and during the study clinical chemical measurements should indicate the development of any pathological lesions. The data derived from sub-acute toxicity studies also help in the design of chronic toxicity studies. Attempts are usually made in sub-acute toxicity studies to identify a no-observed effect level, taking data from other tests into consideration.

Chronic Toxicity Tests

These tests involve lifetime exposure of animals to the compound of interest. As with sub-acute toxicity tests the chronic toxicity test will terminate with a pathological examination and there may also be clinical chemical measurements made throughout at intervals. These clinical chemical measurements can indicate the development of pathological changes which can then be detected at post-mortem. Changes in other simple measurements such as body weight and food and water intake may also indicate adverse effects. Chronic toxicity studies are important for drugs administered over long periods of time, for food additives to which we may be exposed for our whole lifetimes and for environmental and industrial chemicals where we may be exposed to low levels for long periods.

For all three types of toxicity test, selection of dosages, species, strain of animal, route of exposure, parameters measured and many other considerations are vitally important. These considerations will clearly be influenced by the particular type of chemical, expected circumstances of exposure and the regulations of the countries in which the substance is to be used. For details of these toxicity tests the reader is referred to the texts given in the Bibliography.

The requirements of the New Substances Regulations in the UK serve to illustrate the range of physico-chemical, toxicological and ecotoxicological studies that may be required. Under these regulations the amount of testing required depends upon the amount of the substance produced but the minimum requirements are shown in Table 11.2. In addition, teratology, fertility, further subchronic, carcinogenicity and chronic toxicity studies may be required depending on the amount of the compound produced and the results of other tests. It may also be necessary to repeat some of the studies already carried out but using alternative routes of administration or a different species of animal for instance. Similarly ecotoxicology studies may also need to be increased to include prolonged toxicity studies in *Daphnia* and fish, effects on higher plants and determination of bioaccumulation in fish and possibly other species. The tests described are the basic ones required and serve to illustrate the principles involved. However, other tests will also be required such as teratogenicity and other reproductive studies, carcinogenicity, mutagenicity, irritancy and skin sensitization.

Table 11.2. Summary of major information required for a new chemical substance*.

Identity	**Toxicology studies**
name/trade name	acute toxicity (oral/inhalation/cutaneous)
formulae (empirical/structural)	skin and eye irritancy
composition	skin sensitization
methods of detection/determination	subacute toxicity (28 days)
	mutagenicity (bacterial and non-bacterial)
Uses and Precautions	**Ecotoxicological studies**
proposed uses	toxicity to fish
estimated production/importation	toxicity to *Daphnia*
handling/storage/transport methods and precautions	degradation data (BOD, BOD/COD)
emergency measures	
Physico-chemical properties	**Possibility of rendering substances harmless**
melting point	for industry
boiling point	for public
relative density	declaration concerning the possibility of unfavourable effects
vapour pressure	proposed classification and labelling
surface tension	proposals for any recommended precautions for safe use
water solubility	
fat solubility	
partition coefficient (octanol/water)	
flash point	
flammability	
explosive properties	
auto-flammability	
oxidizing properties	

* This represents the minimal information required for a new substance under the UK and EC regulations. Taken from *Medical Information* (1985) **10**, 123–127, Woodward and Tomlinson.

Reproductive studies determine the effect of the compound on the reproductive process. Thus, teratogenicity tests examine the effect of the compound on the development of the embryo and foetus. These may be detected as gross anatomical abnormalities in the new born animal or may be more subtle effects such as changes in behaviour. The effect of the compound on the fertility of both male and female animals may also be determined in reproductive toxicity tests. Data from other tests may also be relevant, such as pathological evidence of testicular damage which might additionally be detected as a decrease in male fertility.

Mutagenicity tests determine whether the compound has potential to cause genetic damage and so induce a mutation in germ cells and somatic cells. Such tests indicate whether a compound may have the potential to induce cancers. Mutagenicity tests are carried out in bacteria and cultured mammalian cells *in vitro*. *In vivo* assays include the micronucleus test and the dominant lethal assay (see Bibliography for details).

Carcinogenicity tests may also be required, especially if the mutagenicity tests are positive. The compound is given for the life time of the animal, administered either in the drinking water or diet. The appearance of tumours at post-mortem or perhaps before the animal dies are detected from histopathological studies of sections of tissues from the major organs.

Irritancy and skin sensitization tests may also be required, especially for industrial chemicals and pesticides. Irritancy tests are usually carried out on rabbit skin or eyes. The skin sensitization test is normally carried out in the guinea pig and a positive result indicates that the compound has the potential to cause contact dermatitis in humans. Some compounds may also cause pulmonary sensitization but there is no reliable animal model for this effect. Consideration of the toxicity data may suggest that further studies be carried out, such as an investigation to show that an effect is peculiar to a particular species and therefore not relevant to man.

Toxicity tests are normally either carried out by the company producing the compound or a contract research laboratory or a combination of both. The conduct of the toxicity and ecotoxicity studies should conform to certain guidelines, such as those issued by the Organisation for Economic Cooperation and Development (OECD). These guidelines are often enshrined in national regulatory requirements such as those in the UK and USA. Toxicity tests also now must be carried out in compliance with a system known as Good Laboratory Practice (GLP), which governs every aspect of the conduct of studies including the reporting of results. This system was introduced to ensure that toxicity tests are competently carried out and that data is not fabricated, following a notorious situation which arose in the USA.

As well as the requirements of regulatory agencies toxicity data may also have other uses. Indeed, the data may be life saving in cases of human and animal poisoning. For example, animal studies on cyanide toxicity provided data which was useful in the treatment of poisoning with cyanide. The absence of any toxicity data on methylisocyanate probably hampered the efforts of rescue workers and clinicians at Bhopal in India after the massive disaster where methylisocyanate leaked from a chemical plant there. Basic studies on paracetamol toxicity led directly to the use of an antidote which has proved extremely successful and life saving. Attempts to understand the mechanisms underlying the toxicity of compounds will allow better prediction of toxicity and also better design of tests to discover toxic potential.

Risk Assessment and Interpretation of Toxicological Data

At least 65 000 chemicals are currently produced in the USA with 500–1000 new chemicals added each year. In the past, perhaps chemicals were too readily produced and used without due care and attention. Rachel Carson in her book, *Silent Spring* showed the risks of such actions. The general public is now very suspicious of all chemicals and there is perhaps an exaggerated fear of poisoning from chemicals in the environment and a belief that all chemicals are hazardous. Regulation has been introduced in many countries in response to this public fear and pressure. Clearly regulation is necessary, but where possible guidelines should be issued rather than strict rules for the assessment of every case in the same way. A major problem with toxicological data is the assessment of hazards and the subsequent calculation of risks and estimation of risk versus benefit.

'Risk is a measure of the probability that an adverse effect will occur.' This may be absolute risk which is the excess risk due to exposure, or relative risk which is the ratio of risk in the exposed to the unexposed population. For a chemical being considered as a toxic hazard, risk is the expected frequency of undesirable effects arising from exposure to that chemical and is a function of the intrinsic toxicity and the dose or exposure level. The exposure level is determined by the duration, frequency and intensity of exposure, which will in turn depend on the circumstances of exposure in a particular environment. For example, in a factory manufacturing a particular chemical the production workers may suffer continuous moderate exposure whereas maintenance workers may be subject to much higher concentrations periodically during work on a reaction vessel. Workers in other parts of the factory and office workers on the site may have only negligible exposure and perhaps be only at risk from an accidental leak. Depending on the disposition and toxicity of the particular compound, the risk to the production worker with a greater total exposure may be less than that to the maintenance worker exposed to very high concentrations. Alternatively, if the compound causes allergic reactions then continuous exposure will be more important and the production worker may be more at risk. Therefore, risk assessment involves first an identification of the hazard, followed by an estimation of the exposure level and frequency, and then a knowledge of the *in vivo* disposition, toxicity and dose response relationship.

The assessment of exposure and dose is critical in risk assessment but can be very difficult to estimate in a human population as exposure may be affected by many factors. For instance, lifestyle varies among humans, the exposure may vary in frequency and it may be periodic. Such factors are difficult to simulate. In manufacturing plants the workers may be monitored for exposure to a substance by measuring its concentration in their blood or urine. Alternatively their exposure may be monitored by the use of personal or environmental metering systems.

In the general population, however, this is much more difficult. So although currently available analytical methods are often able to very accurately determine minute levels of toxic compounds in the environment, determination of the exposure of humans to those compounds is much more difficult and much less precise.

The accuracy of measurement has increased over the years and so has our ability to detect ever smaller amounts but this may lead to paranoia over insignificant levels of substances.

The science of toxicology involves observing the qualitative and quantitative effects of compounds in biological systems *in vivo* and *in vitro*. The art of toxicology is the use of this data or a limited database to predict the likelihood or probability of occurrence of a toxic effect. This requires extrapolation between species and between doses or levels of exposure.

Acute toxic effects are usually easier to deal with than chronic toxic effects as it is generally accepted that there will be a 'no observed adverse effect level' or NOAEL. This can be derived from the dose-response relationship. So it is possible to derive an acceptable daily intake value or ADI for a food additive,

for example, a therapeutic index in the case of a drug or a TLV (MEL) for an industrial chemical (see Chapter 2 or Glossary for a definition of these terms). Chronic toxicity and especially carcinogenicity and teratogenicity are more difficult to deal with. Theoretically, a single 'hit' or reaction of a compound or its metabolite on the crucial part of one DNA molecule might be sufficient to initiate a cancerous change. However, the chances of one molecule reaching this target site are probably small for most compounds. This will be determined by the potency, absorption, distribution and metabolism of the compound and these will affect its ability to reach and damage DNA. The capacity of the particular cell to repair such damage will also be crucial. Effectively, therefore, there may be a threshold dose for a carcinogen but it is difficult to determine in mammals *in vivo* because the crucial biochemical changes at the cellular level are currently difficult if not impossible to detect. Consequently, bacterial assays such as the Ames test are used which detect such mutagenic changes. The results from animal carcinogenicity testing studies are particularly hard to assess as it is necessary but difficult to show an increased frequency of tumours in a small population such as those used in animal cancer studies, in which there may already be a significant incidence of some types of tumours. There is a practical, statistical limit which determines the incidence or frequency of occurrence of a cancer which can be detected. For example, using 1000 animals it is necessary for more than five animals to be affected by cancer for the effect to be detected at the 99 per cent confidence level; but an incidence of five cases in 1000 test animals if extrapolated to man would translate into over 1 million cases of cancer in a population the size of that of the US. To use even larger numbers of animals would be impractical, extremely expensive, and challenged on ethical (animal rights) grounds. So assessing cancer risk from carcinogenicity studies is very difficult and those conducting and assessing the tests tend to err on the side of caution. One way around the dilemma of low incidence is to increase the doses used in the animal tests on the assumption that the dose-response is linear and so extrapolation backwards is possible. This has given rise to various models but estimates from these models vary; the precision of the mathematical model is largely irrelevant if the quality of the original toxicological data is poor. There may be large margins of error and uncertainty. Unfortunately the public may take the exposure limits and similar data issued at face value or alternately disbelieve them completely. Consequently, doses close to the Maximum Tolerated Dose (MTD) are used in carcinogenicity testing despite the problems of dosedependent metabolism, dose-dependent kinetics, and the possibility of other pathological effects influencing the carcinogenicity. This approach is contentious, however, as carcinogens may show dose dependent metabolism and with weak or equivocal carcinogens such as saccharin (see Chapter 6) and especially non-genotoxic carcinogens this may be crucial to the interpretation of the carcinogenicity data. That is, large doses of a compound may be metabolized in a quantitatively or qualitatively different manner to that of the expected dose or exposure level. Consequently, a compound may only be carcinogenic under those extreme dosing conditions. For example, the industrial chemical hydrazine is a weak carcinogen after high exposure or dose levels. It also

causes DNA methylation, a possibly mutagenic event which might lead to cancer but this methylation only occurs after large, hepatotoxic doses. The implications of this are that the acute toxic effect is in some way involved in the DNA methylation and that also the acute effect is necessary for the development of the cancer.

Extrapolation between species is also a problem in risk assessment and the interpretation of toxicological data. For example, one question that arises is 'which species is the extrapolation to be made from, the most sensitive or the one which in terms of response or disposition of the compound is the most similar to man?' The species or strain used in a particular carcinogenicity study may have a high natural incidence of a specific type or types of tumour. The assessment of the significance of an increase in the incidence of this tumour and its relevance to man can pose particular problems. Therefore, risk assessment from carcinogenicity is fraught with difficulties, possibly more than any other type of toxic effect.

For acute toxic effects the dose response is often clear cut and allows a NOAEL to be estimated. However, the biology of the toxicity study must always be taken into account and a too exaggerated reliance on statistics must be avoided. Because of the problems of inter species extrapolation and interpretation of low incidences of tumours, risk assessment may give rise to widely disparate quantitative values. For example, for saccharin the expected number of bladder cancer cases in the USA over a 70-year period due to daily exposure to 120 mg was estimated as between 0.22 and 1.144×10^6! Therefore, in the risk assessment of a particular compound other factors become important such as the likely and reasonable human exposure but in the USA the strict rules of the Delaney clause make this difficult (see Glossary for definition of Delaney Clause).

The incidence of a toxic effect may be measured under precise laboratory conditions but extrapolation to a real life situation to give an estimate of risk involves many assumptions and gives rise to uncertainties. The risk assessor has to decide which are plausible answers to questions when in reality there are either no scientific answers or these answers are obscure. Risk assessment involves questions such as which model of the dose-response curve to use for extrapolation. Pharmacokinetic, mechanistic and metabolic data will all affect this.

How should the real human exposure be estimated from limited data? The problems of doing this tend to lead to the worst case estimate and so estimates of risk will tend to exaggerate the risk to human health.

For a new chemical substance human data is not available and toxic effects in man cannot be verified by direct experiment and so extrapolation from the results of animal studies is essential. Of course the objective is to have as large a margin of safety as possible. However when there is conflicting data does one use the single positive result or the 'weight' of all the data? Inflated estimates of exposure may occur. Epidemiology may be useful for compounds that have been used for some time. Indeed, many compounds have never undergone a full range of toxicity tests (an estimated 70 per cent in USA) and it would clearly be an enormous task to test all such compounds. Consequently, a reliance on epidemiology is unavoidable.

Risk assessment is followed by risk management and this includes a consideration of the benefits which inevitably involves politics and economics. Risk assessment itself may also be influenced by these factors.

Conclusions

As yet, toxicologists only partially understand the mechanisms underlying relatively few toxic effects of chemicals. Consequently the assessment of risk to man will remain difficult and uncertain. The limitations need to be borne in mind by the public, by industrialists, economists and regulatory officials, but also by toxicologists themselves.

Perhaps the public expects too much from scientists in general and toxicologists in particular. Toxicology cannot provide all of the answers the public often demands as they are beyond current science. The public may demand absolute safety but this is an impossible dream. One of the duties of the toxicologist is to make sure the limitations are understood.

Perhaps the real crux of the problem of interpretation of toxicological data in the light of increasing and widespread exposure of humans to chemicals is the assessment of risk versus benefit. Although the public may not always be aware of the fact that chemicals confer benefits on society, and that there is a greater or lesser risk attached to their use, the benefits may be hard to quantify and compare with the risk. However, just as we take a quantifiable risk when we drive a car because its use is convenient and maybe essential, then we should apply similar principles to the chemicals we use. Unfortunately the risks and benefits may not always be equally shared, with one section of society reaping financial benefits while another risks the adverse effects.

Questions

1. What factors need to be taken into account when designing safety evaluation studies?
2. Write short notes on the following:
   (a) acute toxicity tests;
   (b) sub-acute toxicity tests;
   (c) chronic toxicity tests.
3. Discuss the difficulties inherent in the interpretation of toxicological data for risk assessment.

Bibliography

Ballantyne, B., Marrs, T. and Turner, P. (Eds) (1993) Regulatory toxicology, part six, vol. 2, *General and Applied Toxicology,* Basingstoke, UK: Macmillan.

Griffin, J.P. (1985) Predictive value of animal toxicity studies, *ATLA,* **12**, 163.

Hayes, A.W. (Ed.) (1989) *Principles and Methods of Toxicology,* 2nd edition, New York: Raven Press.

Heuvel, M.J.Van Den, Dayan, A.D. and Shillaker, R.O. (1987) Evaluation of the BTS approach to the testing of substances and preparations for their acute toxicity, *Human Toxicology,* **6**, 279.

Homburger, F. (Ed.) (1983–) *Safety Evaluation and Regulation of Chemicals,* 3 vols, Basel: Karger.

Lu, F.C. (1991) *Basic Toxicology,* 2nd edition, Washington, DC: Hemisphere.

Merrill, R.A. (1991) Regulatory toxicology, in *Cassarett and Doull's Toxicology,* Amdur, M.O., Doull, J. and Klaassen, C. (Eds), 4th edition, New York: Pergamon Press.

NIEHS (1987), Basic research in risk assessment, *Environmental Health Perspectives,* **76** (Dec).

Roberts, C.N. (Ed.) (1989) *Risk Assessment—The Common Ground,* Eye, Suffolk: Life Science Research.

Roloff, M.V. (Ed.) (1987) *Human Risk Assessment. The Role of Animal Selection and Extrapolation,* Philadelphia: Taylor & Francis.

Scala, R.A. (1991) Risk assessment, in *Cassarett and Doull's Toxicology,* Amdur, M.O., Doull, J. and Klaassen, C. (Eds), 4th edition, New York: Pergamon Press.

Wilkinson, C.F. (1986) Risk assessment and regulatory policy, *Comments on Toxicology,* **1**, 1–21.

Zbindon, G. and Flury-Reversi, M. (1981) Significance of the LD_{50} test for the toxicological evaluation of chemical substances, *Archives of Toxicology,* **47**, 77.