CHAPTER 7

ANIMALS IN TOXICOLOGICAL TESTING

Introduction

7.1 An assessment of the use of animals in testing drugs and cosmetics requires some understanding of the nature of toxicological research and the regulatory framework within which it currently operates. A brief account of the scientific basis of toxicological testing is given before regulatory issues are considered.

7.2 There are two basic approaches to the science of toxicology - the mechanistic and the descriptive. Mechanistic toxicology is the study of the chemical processes by which a toxic effect occurs. It relies on techniques developed by physiologists, biochemists and analytical chemists to monitor these processes. Mechanistic toxicology is research oriented. It provides the basis for the design and interpretation of descriptive tests and is essential to the development of testing methods that could replace whole-animal testing.

7.3 Descriptive toxicology relies on the information provided by pathology, statistical analysis, physiology and pharmacology. It involves, for example, evaluation of changes in the appearance of an organ or its cells, the appearance of tumours or signs of irritation. An understanding of the exact nature of the processes by which the toxic effects occur is not necessarily required. Regulation of chemicals requiring testing for toxicity relies largely on descriptive toxicology.[1]

7.4 Many toxicological tests currently require the use of whole animals. The most appropriate animals are those which predict the human response to a specific substance most accurately. The choice of animal is influenced not only by the similarity of the animal's organism or biochemical mechanism of concern to the testing authority to that of humans, but also of such factors as the convenience of breeding, the extent of pre-existing knowledge of the species, species lifespan, ease of handling of species under experimental conditions, cost of purchase and maintenance, litter size and gestation period.

Testing Strategies

7.5 In most toxicity tests the substance being tested is administered by the same route as occurs in the course of accidental exposure or use by humans. On occasions the palatability, solubility, stability, and volatility of a substance determines the routes that are feasible.

7.6 The dose levels employed in a testing programme need careful consideration. If the dose is so large that many animals die before the end of the test, it will not be possible to detect long-term effects. If the dose is representative of human exposure levels it may not produce detectable effects without the use of an excessively large number of animals over a long period of time.

7.7 The test design must be statistically sound if valid results are to be obtained. Factors influencing the number of animals needed for a given test include:

(1) the need to allow for unexpected death and illness in the test group;
(2) variability in the sensitivity of individual animals to the substance being tested; and

(3) the need for an untreated control group to provide information on the background incidence of disease against which the incidence in the groups discussion in being tested can be assessed.[2]

Drug and Chemical Testing in Australia - Regulatory Requirements

7.8 The Commonwealth has no direct constitutional power to regulate drugs and chemicals. It does have, however, some indirect means of control by virtue of its constitutional power over imports. This power is exercised through the Customs (Prohibited Imports) Regulations. Under these Regulations, the Commonwealth can ban the importation of particular drugs or ingredients. However, the Commonwealth cannot prohibit drugs which are manufactured from ingredients sourced within a State and sold within the same State. State legislation covers the extent and nature of testing required before marketing of therapeutic goods and agricultural or industrial chemicals.

7.9 Through the co-operation of Commonwealth and State Governments, there is national co-ordination of the safety assessment and control of chemicals. A plethora of expert committees comprising Commonwealth and State officers and experts from the industry and universities carry out the assessments and provide advice to government on the control and regulation of chemicals.

7.10 Drugs for human therapeutic use are evaluated by the Australian Drug Evaluation Committee supported by the Drug Evaluation Branch of the Department of Community Services and Health.

7.11 The NHMRC is responsible for the toxicological assessment of most chemicals not designed for therapeutic use, including agricultural chemicals. With regard to agricultural and veterinary chemicals, national co-ordination has been in the

hands of the Co-ordinating Committee on Agricultural Chemicals which has been responsible to the Australian Agricultural Council. The enactment of the Agricultural and Veterinary Chemicals Act 1988 represents an attempt to provide a more co-ordinated approach to the regulation of agricultural and veterinary chemicals. Under the Act an Australian Agricultural and Veterinary Chemicals Council will be established to co-ordinate the evaluation of chemicals proposed for registration in Australia, including assessments of toxicology, human safety, environmental hazard and overall efficacy.

7.12 Plans to regulate industrial chemicals are intended to complement current national arrangements for the evaluation of agricultural chemicals, pharmaceuticals and food additives that are already in place.

7.13 The scheme will be established under Commonwealth legislation although State Governments may decide to enact complementary legislation. In essence, importers will have to notify NOHSC of imports of industrial chemicals and to provide it with enough information for NOHSC to assess the potential health and environmental hazard of the chemicals. The NOHSC will generally rely on data used to satisfy regulatory requirements overseas but will if necessary seek additional information. It is expected that most additional tests will be done overseas.

Cosmetics

7.14 The responsibility for national standards for cosmetics ingredients was transferred in 1987 from the NHMRC Consumer Products Safety Committee (of the then Department of Health) to the new Bureau of Consumer Affairs within the Attorney-General's Department. In 1986 the Consumer Products Safety Committee of NHMRC had set up a Working Party to prepare appropriate standards for cosmetics ingredients. According to the Department of Community Services and Health:

> The Working Party used the general structure of the EEC cosmetics Directive as a working document, but amended the lists of acceptable colours, sunscreens and so on to suit Australian circumstances. Like the European standard, it was proposed that the NH & MRC Standard would not list all possible cosmetic ingredients. Ingredients were to be included in the various Annexes to the Standard on the basis of either a history of safe use or as a result of a toxicological assessment.[3]

7.15 The Committee was told by the Department that in preliminary work prior to the transfer of responsibility to the Bureau, it was established that many chemicals were being used without toxicological work having been done on them. The Department said that the industry had pointed out that these chemicals had been used without ill effects to consumers. Although the Department did not intend to seek toxicological information on chemicals currently in use, it would have insisted on the submission of toxicological data on new chemicals.[4]

General Issues in Testing

7.16 In discussing its approach to testing requirements, the Department of Community Services and Health made the following points:

> 1. Animal studies are not required unless they will contribute worthwhile information on the new medicine;
>
> 2. Large numbers of animals in any one test are not required providing that the number used will be capable of discerning the problem to be investigated;
>
> 3. The LD50 test as such is not required, being replaced with acute studies to include relevant observations;
>
> 4. Non-human primates were required in some studies but this requirement, as such, has now been deleted;

5. The use of in vitro screening tests is recognized and accepted;

6. The Australian guidelines are very similar to and consistent with many overseas requirements so that any additional animal testing for some medicines is kept to a minimum;

7. Some overseas countries have required a certain amount of animal testing to be repeated in their own country. The Australian Department of Health accepts data generated overseas without any requirement for animal studies to be repeated in Australia.[5]

7.17 Although Australian guidelines are similar to overseas guidelines, there are differences between them. The Australian guidelines are being rewritten to achieve a greater harmonisation of requirements with overseas countries and international organisations such as the European Community, the Organization for Economic Co-operation and Development and the World Health Organization.

7.18 It should be noted that the demand for further testing caused by a lack of uniformity in requirements does not mean that such tests would be carried out in Australia. A lack of uniformity would be most likely to result in an increased use of animals for toxicological testing overseas.

Toxicological Testing in Australia

7.19 Toxicological tests using live animals are not done on a large scale in Australia. Most tests are conducted overseas where the products are developed. Data from these tests are submitted to Australian authorities in support of applications for registration of products. The former Australian Bureau of Animal Health outlined the main purposes for which testing was conducted in Australia:

> Some chemical evaluation studies are performed in Australia using animals. The purpose of additional testing is to generate data on the performance of the chemical under Australian conditions, e.g. efficacy against local pest species of weeds, insects or internal and external parasites. Local testing is also performed on veterinary drugs to show that the product is safe for the target animal. These tests usually take the form of a medium scale field [trial] where animals are treated with the drug at an elevated dose rate to assess the safety in situations of accidental overdose. Rarely, however, do such tests involve the estimation of the LD50 in the target animal.[6]

7.20 In its submission the Cosmetic Toiletry and Fragrance Association of Australia stated:

> In this country, the cosmetic industry consists largely of subsidiaries of overseas companies and as a consequence, most research resulting in toxicological validation is carried out abroad, with an insignificant level of safety testing locally, carried out by independent toxicological laboratories.
>
> Australian manufacturers of cosmetics have access to all relevant hard copy and computerised data banks through the CTFAA or from their principals abroad, thus enabling them to eliminate almost all animal testing for cosmetics in this country.[7]

7.21 There was, however, a distinct reluctance or possible inability of the relevant industry associations to supply the Committee with statistics from their members on the actual extent of use of animals for toxicological testing within Australia.

7.22 The Department of Community Services and Health provided indirect evidence on the extent of animal use for toxicological testing within Australia. Dr Imray of the Department stated:

> There has been no data submitted that I have seen in the time that I have been with the Department that has ever been generated anywhere other than in the major toxicology

> contract laboratories overseas or through company laboratories overseas. In all of the submissions that I have seen, I have never seen data generated in Australia.[8]

7.23 Although the Committee accepts that commercial toxicity testing using animals for regulatory purposes conducted within Australia is minimal in extent, it is of the view that a willingness by commercial enterprises to be more open with the public would do much to assuage public concern.

National Biological Standards Laboratory

7.24 The numbers of animals used in the National Biological Standards Laboratory (NBSL) is contained in Table 7.1. Its use of animals was explained as follows:

> 3.1 NBSL testing of products is intended to assess their quality, safety and efficacy. Quality is a wide ranging concept covering aspects of conformity with specifications, fitness for intended use and consistency of production ...
>
> 3.2 NBSL testing of products for quality is, wherever possible, performed using chemical or physical methods. These methods generally offer advantages of speed, precision and economy over biological methods. However they are usually only generally applicable to products whose chemical or physical characteristics are known. Many biological products such as vaccines, hormones, enzymes and blood products are heterogeneous mixtures of complex compounds whose chemical and physical characteristics have not been established. It is usually necessary to perform at least some biological tests on these types of products when assessing their quality.
>
> 3.3 Biological methods can range from in vitro methods such as biochemical techniques, immunological techniques, cell culture techniques and isolated cell or organ culture

techniques through to in vivo techniques involving embryonated eggs or whole animals. Where in vitro methods cannot adequately assess a characteristic of a product a whole animal technique must be used.

3.4 Safety tests in particular often require the use of whole animals. Safety from the NBSL viewpoint usually refers to lack of adventitious contamination with toxic substances rather than to the inherent toxicity of the product.[9]

Table 7.1: The Numbers of Small Animals Issued to Users from NBSL 1975-1989

Period	Mice	Rats	Guinea Pigs	Rabbits	Chickens
1975	77,610	1600	1490	*	*
1976	62,230	900	1370	*	*
1977	49,200	980	2030	*	*
1978	87,760	1030	3520	*	*
1979	75,640	890	2960	*	850
1980	96,470	830	4510	*	1254
1981	63,060	230	3440	410	1032
1982	52,950	520	3260	405	1573
1983	51,650	290	2300	400	1269
1983-84	44,420	600	2630	370	1138
1984-85	35,900	1450	2130	280	1026
1985-86	32,000	530	2190	225	614
1986-87	27,400	370	1590	300	1020
1987-88	18,000	1560	1410	370	925
1988-89 Prorata	24,000	2280	1704	370	656

* No records available
SOURCE: Evidence, p.S8045

7.25 Detailed statistics on the use of animals in toxicological tests, including those conducted in course of research as well as those done to satisfy regulatory requirements, are currently only available for Victoria.

7.26 Because Commonwealth departments or statutory authorities involved in animal experimentation are not registered or licensed under the relevant Victorian legislation such statistics would not include, for example, those animals used by the Commonwealth Serum Laboratories or the CSIRO Division of Animal Health. The Victorian Government publishes annually the number of animals used in the State for toxicological and related pharmacological research and testing. Figures from that statistical report for the five years 1982-83 to 1986-87 are shown in Table 7.2.

7.27 Table 13 in of the Victorian report contains a breakdown of the figures reproduced in Table 7.2 by types of tests including a category for tests performed to meet the NHMRC Toxicological Data Requirement. Because of difficulty in reconciling the figures in these two tables for most categories in most years it was not possible to use the disaggregated figures with any high degree of confidence. Hence they have not been used in this report.

Table 7.2: Number of Animals Used in Toxicological and Pharmacological Testing in Victoria 1982-1987

	Toxicity Tests	Teratological Tests	Distribution Metabolism excretion and residue tests of substances	Total
1982-83	2,927	100	22,704	25,731
1983-84	6,097	643	11,983	18,723
1984-85	832	124	3,676	4,632
1985-86	3,885	3	5,692	9,580
1986-87	7,787	312	3,985	12,084

SOURCE: Department of Agriculture and Rural Affairs, Bureau of Animal Welfare. Statistics of Animal Experimentation in Table 4; 1982-83, 1983-84, 1984-85, 1985-86 and 1986-87 reports.

The Role of Animals in Toxicological Testing - Rationale and Alternatives

7.28 Consideration of animal use in toxicological testing brings into focus a major conflict in public expectations. On the one hand the public wants to minimise the risks to humans, animals and the environment arising from the development and widespread use of chemicals. On the other hand there are undoubtedly public reservations about or opposition to the use of animals for toxicological testing.

7.29 The rationale for the use of animals in toxicological testing arises from the responsibility of the appropriate authorities at least to ascertain the risks associated with the

use of chemicals by the public. This prima facie responsibility is regarded by government as overriding, but not negating moral responsibility for the welfare of animals.

7.30 It appears that whole animal tests are unlikely to be completely replaced by non-animal or in vitro methods[10] because in vitro tests cannot reproduce the functional and structural complexity of the intact animal. In vitro tests cannot preserve the diversity of mechanisms for toxicity and detoxification that exist in living organisms. At each successive level of biological organisation properties appear which are not evident or even present at less complicated levels of organisms or systems.

7.31 Three issues about testing whole animals were raised by ANZFAS in its submission.

7.32 The first is the difficulty of extrapolating results from non-human species to humans.[11]

7.33 ANZFAS drew attention to the following cautionary note in the OECD Guidelines for Testing of Chemicals:

> There is no experimental laboratory species which is identical to man in terms of structure or metabolism. There are obvious resemblances and similarities in function between man and other animal species, but even in the case of man's fellow primates, these are not such that straightforward extrapolations from animal tests to man are possible. The interpretation of animal test results in the assessment of possible human health hazard remains a matter of skilled judgement.[12]

ANZFAS went on to say:

> While authorities require the use of at least two mammalian species for the testing of one substance, the problem of extrapolation is

> increased two-fold. Not only are there differences between two species, but also between animals of the same species within one laboratory.[13]

It then referred to the work of Zbinden and Flury-Roversi:

> It (the LD50) can vary markedly from one animal species to the other, and within one species of laboratory animals the numerical value of the LD50 determined experimentally is influenced by a large number of factors.[14]

7.34 Mr Van Rijswijk of the Australian Veterinary Chemicals Association (AVCA) responded to the criticism:

> Whenever we use a test animal to work out the effect of a chemical or a drug we rely on that test animal to parallel somehow what happens in our bodies. Because the biology is different - we are not rabbits or rats - that model is only a model, it is not a perfect duplication of the human system ... we can test thousands of animals and we can test many different species of animals but we are never going to duplicate what that chemical does inside our body. That is recognised by toxicologists. If that is the case, adding more and more animals to that list of testing is not going to give us much more information that really duplicates what is happening inside us.[15]

7.35 The scientific literature suggests that while the extrapolation of the fact of toxicity to humans on the basis of animal studies is a reasonable working assumption, caution is needed in extrapolating the form of toxic action based on those studies.

7.36 In a symposium held in November 1982, Ralph Heywood commented:

> Surprisingly, there has been little effort to examine the qualitative predictability of human side-effects from animal studies.

> Occasionally, general papers have suggested that predictions are unreliable ...
>
> In the absence of better data, it must be concluded from these limited studies attempting to extrapolate data between laboratory animal species, and between laboratory animals and man, that there is no reliable method of predicting what type of toxicity will develop in different species in response to the same compound.[16]

7.37 ANZFAS emphasised the extent to which the logistics of testing influence the choice of species to be used in the tests. It drew attention to the OECD Guidelines for Testing of Chemicals which pointed out that such factors 'as ease of breeding or purchasing, animal husbandry, speed of growth/development and handling under the experimental conditions' are considerations in choosing the species. ANZFAS went on to state:

> For acute oral, dermal inhalation studies the rat is the most frequently used species. The extensive use of rodents in toxicological studies would appear to be perhaps nearly as much a function of the logistic requirements referred to above as any particular superiority in predicting the likely human response.[17]

7.38 ANZFAS also raised concerns about the extent to which data from different testing laboratories were comparable.[18] As an example of the deficiencies in this area it cited the example of the difficulties encountered by the FRAME Cytotoxicology Research Project which was completed in 1985. The Project was involved in the development of non-animal alternatives for cytotoxicology tests. In order to carry out validation studies of the non-animal tests, toxicity data on 100 chemicals was sought, against which the in vitro methods could be measured.

> The toxicology data are often not strictly comparable, being developed in different laboratories using different species or different protocols. The reports describing the toxicology data are often inadequate and

> the data itself may present inherent problems of interpretation, which would result in legitimate differences in assessments of their toxic effects by different toxicologists.[19]

Alternatives

7.39 In Chapter 5 the discussion on alternatives to the use of animals in experiments included those techniques or methods that replace the use of laboratory animals, reduce the number of animals required or refine the existing procedure or technique so as to minimise the amount of pain or distress endured by the animal.

7.40 The major developments in alternatives in toxicity testing to date have come from the reduction of the number of animals required for each test and the refinement of the test procedures to reduce animal suffering.

7.41 Most of the alternatives to the use of animals in testing fall into one of the following four categories:

(a) the continued but modified use of animals;
(b) the greater use of living systems;
(c) the greater use of non-living systems; and
(d) the further development of computer simulation.

7.42 The continued but modified use of animals includes alleviation of pain and distress through analgesics and less intrusive methods, substitution of cold-blooded for warm-blooded vertebrates, co-operation among experimenters in the shared use of animals, and a statistical design of experiments which enables reliable information to be obtained with fewer animals than were used previously. This can be achieved by reducing the number of animals used as controls, by using the same group as controls for several simultaneous experiments, avoiding duplication of testing by storing data, reducing pain and distress by changing procedures, refining the end point of a study.

7.43 The greater use of living systems includes micro-organisms, invertebrates and the in vitro culture of organs, tissues and cells. The advantages and disadvantages of this approach have been summarised by the OTA:

> Although animals are still required as a source for these in vitro systems, the animal would experience distress for a much shorter time, and perhaps less distress overall, than occurs with whole-animal testing because it would be killed before any experimental manipulations were carried out. Occasionally, different cells, tissues, or organs from the same animals can be used for different investigations. In addition, many fewer animals would be required for a given test, in part because variability in the toxic response is smaller than it is with whole-animal tests and in part because one animal can be used for multiple data points, further reducing variability. The fact that human tissues sometimes can be used confers an additional advantage because the need for extrapolation from animal data is obviated.
>
> These isolated components also have disadvantages. They are usually unable to produce the complete physiologic responses of a whole organism. The components often become undifferentiated and lose their ability to perform their special functions when isolated from the organism, particularly when the sample is broken up into its constituent cells, and even more so when the cells replicate. Another disadvantage is that the effect of the route of exposure, a variable that can have profound effects on test results, is often impossible to determine.[20]

7.44 Micro organisms such as bacteria and fungi are principally used to measure genotoxic effects. They can be cultivated more easily and quickly than most animal or human cells. Their genetic makeup is simple and changes in it are relatively easy to detect.

7.45 Invertebrates such as insects offer the greatest variety of models. The fruitfly Drosophila Melanogaster is best understood and has been used for detecting teratogenicity, mutagenicity and reproductive toxicity. The sea urchin has also been widely used for screening for mutagenicity, teratogenicity and reproductive toxicity.

7.46 The greater use of non-living systems includes epidemiologic data bases of human diseases and causes of death and physical systems that mimic biological functions. However, these cannot be relied on for prospective toxicity testing of drugs or chemicals.

7.47 Whole animals have been replaced with analytical chemistry for tests involving detection of a substance or measurement of potency or concentration, such as vaccines, anti-cancer drugs and vitamins.

7.48 There is the further development of computer programs that simulate biological functions and inter actions. Sophisticated mathematical models have been developed which predict biological responses to the drug and hence toxicity on the basis of physical and chemical properties, structure and available toxicological data. The major limitation of these models is the lack of understanding of the mechanisms by which toxic effects occur.

7.49 In considering the impact of alternative tests the Department of Community Services and Health commented:

> ... the general consensus of scientific opinion at present appears to be that, on the basis of current knowledge, no single in vitro (or for that matter, alternative) test will directly replace any one in vivo test. A combination or battery of in vitro tests will probably be required for most if not all toxicological parameters. As a result of

> putting a chemical through such a battery of tests, it may still be necessary in some cases, depending on the results, to test it on whole live animals to obtain final confirmation of the nature of potential toxicity. One example of particular concern because of its importance is carcinogenesis, which is a complex process, unlikely to be shown up in a single in vitro assay. Thus it is likely that a selected battery of short-term assays, including both in vivo tests, and in vitro tests in bacterial and mammalian cells, will be needed to screen chemicals for their potential to cause genetic effects and carcinogenicity.[21]

Development of Alternatives in Australia

7.50 Probably because of the small amount of toxicity testing actually undertaken in Australia little work has been done to develop non-animal toxicological tests by Australian scientists. Most of the developments have been within NBSL and are listed in its submission to the Committee.[22]

Specific Toxicological Tests

The Draize Test

7.51 The Draize test is designed to test the irritation to eyes of chemical compounds. It has been criticised on the following grounds:

(a) it can only provide a pass/fail answer and lacks fine discrimination, i.e. it does not provide useful data on degrees of irritancy;

(b) because of differences between human and rabbit eyes its applicability must be in doubt (i.e. it is an unsatisfactory model for human eye irritation comparability); and

(c) there are questions about the comparability of results in routine testing from different laboratories.[23]

7.52 As far as the Committee can determine, the Draize test has been little used in Australia. Among the institutions surveyed by the Committee only the Department of Pharmacology within the University of Melbourne had conducted Draize tests in the period since January 1980. Over the period 1980-1984, 216 Draize tests had been conducted within the Department. Each involved three rabbits and according to the University, were done using the most recent modifications to the test.[24]

7.53 Following a strong campaign by animal welfare organisations in the United States, funding was provided by firms in the cosmetic industry to develop in vitro alternatives to the test. Rowan noted that the response to the availability of funding demonstrated:

> ... that the availability of funding is a potent stimulus to thought. When scientists learned of a possibility of research support to develop an alternative to the Draize test a number of speculative and creative proposals were produced and circulated.[25]

7.54 According to the OTA Report, the current scientific view is that no single alternative is likely to be adequate but that a battery of in vitro tests may be a useful replacement.[26]

7.55 In vitro methods to test for irritation are under development. One promising bioassay for tissue irritation makes use of the chorioallantoic membrane of the chick embryo. Another alternative involves testing whole eyes in vitro. This method has particular appeal when cow eyes are used because of their ready availability from abattoirs.

7.56 The OTA Report, in summarizing the current state of research into alternatives to this test, said:

> Several types of cell cultures have been used in developing an in vitro test for eye irritation. The cells used are rabbit and human corneal cells, mouse and hamster fibroblasts, human hepatoma cells, and mouse macrophages.
>
> ... Rapid progress is being made in the development of techniques, but none can be considered validated at this time.
>
> To date, little work has been done on in vitro replacements for skin irritancy testing. However, the growth of skin in tissue culture is of interest for treating burn victims, and it is expected that culture techniques currently being developed for that purpose can be used in testing methods. In addition, it has also been suggested that suitable specimens can be obtained from cadavers and surgery and from judicious use of human volunteers.[27]

7.57 Draize tests using small dose volume and direct corneal application are being validated currently by Proctor and Gamble. They are of the view that this modified form of test is more accurate in predicting human experience and less stressful to animals.[28]

7.58 Although the Draize test remains in use, a reduction in animal suffering and the numbers of animals used could be achieved by:

(a) not testing substances with physical properties known to produce severe irritation.
(b) screening out irritants using in vitro or less stressful tests.
(c) using smaller volumes of the test substance to reduce trauma and enable dose response studies to determine safety margins; and

(d) the use of local anaesthetics where it is necessary to test substances that cause pain and irritation in the rabbit.

7.59 Some scientists have rejected the use of anaesthetics because they deprive the animal of its natural defence mechanisms, such as blinking.

7.60 The Committee regards the efforts to develop alternatives to the Draize test as an encouraging example of what can be achieved by co-operation among animal welfare organisations, scientists and industry when appropriately targeted funding is provided.

LD50 Test

7.61 The LD50 test is a general measure of toxicity which determines the dose which will kill 50 per cent of the target group of animals.

7.62 The LD50 test has been the subject of widespread criticism by animal welfare organisations. Its usefulness has also been called into question by toxicologists.[29] The figure derived from the test procedure is variable and can be affected by the species, as well as the strain of species used, diet, microbiological status of the animals, the ambient temperature, time of the year and social factors such as the number of animals per cage.

7.63 Fourteen institutions out of those surveyed by the Committee reported that such tests had been undertaken over the five year period 1980-84. In most cases the number of tests conducted by any specific institution was not large. It was not clear from the answers whether the tests were conducted under contract to manufacturers or were University initiated research.

7.64 The CSIRO during 1986 called for a review of the test. It does not currently use the test but has done so in the past to establish the toxicity of naturally occurring plant or microbial substances suspected of being the cause of domestic animal diseases and to obtain the toxicity of pest control agents for both target and non-target species.

7.65 The design and conduct of LD50 studies by CSIRO staff to establish the effect of 1080 baits on non-target species preceded the establishment of ethics committees within CSIRO. The project was specifically designed to reduce the number of animals used to an absolute minimum. CSIRO argued that although the studies undoubtedly caused pain and suffering, the knowledge gained should enable the use of 1080 in controlling feral animals in a manner which minimises losses among non-target species.

Current Status of the LD50 Test

7.66 A number of professional societies and inter-governmental bodies have taken positions recently on the LD50 Test, including the National Society for Medical Research, the Society of Toxicology, Canada and the British Toxicology Society.

7.67 There is substantial agreement that only in very rare circumstances is the precise determination of the LD50 scientifically justifiable. Procedures that allow the classification of toxicity without the determination of the LD50 (e.g. the limit test) could replace it.

7.68 According to the British Toxicological Society:

> ... acute toxicity tests should be carried out with the objective of examining a few animals in detail rather than many animals for statistical purposes. Thus for example the determination of accurate LD50s would not

> appear to be necessary in the drug industry. Acute toxicity tests with minimal numbers of animals and a full description of toxic signs should be adequate for drug development and registration purposes.[30]

Alternatives

7.69 Tests providing the same information have recently been developed using as few as ten animals: that is, a three-fold to ten-fold reduction.

> The development of an in vitro test system for general acute toxicity will be very difficult. Combining in vitro data with computer modelling would probably be the most promising approach ... it will take much money and many years to develop and validate an alternative which will replace animals in LD50 testing.[31]

7.70 Testing for mutagens, carcinogens and possibly teratogens seems to represent a more promising area for the development of non-animal alternatives.

7.71 According to a presentation to the New York Academy of Sciences:

> In establishing non-animal alternatives, two important criteria must be met:
>
> (1) The alternative test, if implemented on a routine basis, will not result in a health risk to humans greater than that presently permitted by use of the animal model.
>
> (2) The introduction of the alternative test will lead to greater efficiency in the assessment of the particular toxic endpoint(s) than currently available animal models.
>
> In the case of genetic testing, these two criteria appear to be attainable.[32]

7.72 Consider, for example, a comparison of the test performance of short-term tests for genotoxicity with the standard rodent bioassay for identifying human carcinogens. The results show that there is approximate equality between the two tests in making an accurate designation.[33] Neither test is a perfect model but there appears to be no loss in the ability to protect humans from carcinogens when non-animal techniques are used.

7.73 The OECD Ad Hoc Meeting of Experts on Acute Toxicity Testing (Paris 7-11 April, 1986) made the following recommendations that would reduce the number of animals required in LD50 tests and refine the techniques used so as to limit animal suffering:

(a) Acute toxicity test guidelines (i.e. OECD) 401 and 402 should be amended so that:

i) oral and dermal tests are carried out on one sex only (with a subsequent check on toxic response of the second sex).

This should almost halve the numbers of animals used.

ii) the limit test dose by the oral method is reduced from 5000 to 2000 mg/kg which is a more realistic dose.

A limit test which results in mortality needs to be followed by a full acute toxicity test. The proposed reduction in the limit dose will result in fewer limit tests being followed by full tests and thus will achieve an appreciable reduction in the number of animals used.

iii) animals which show severe pain and distress are humanely killed in order to reduce suffering.

> Additionally, three new approaches which reduce numbers of animals required, and/or possible suffering, should be distributed to Member countries for evaluation and to gain experience in their use.

Regulatory Action in Australia

7.74 The Department of Community Services and Health explained the development of its policy on the requirement for the LD50 test:

> At the time of writing of the 1984 Departmental submission, the oral LD50 was required. At that time, although it was seen that the LD50 test was probably approaching obsolescence, on balance it was thought that there were good reasons for continuing with the test for the immediate future.
>
> However, shortly after that submission was forwarded to the Committee, data requirements were reviewed and it was decided that the LD50 should no longer be required. This requirement was therefore removed. In its place was substituted the requirements specified by Dr Imray at the hearing before the Committee.
>
> With regard to therapeutic substances, the submission states: '3.6 The general Australian guidelines do not include the LD50 test ...'
>
> The Department's policy, therefore, is that the LD50 test is not required. As stated at the hearing before the Committee, this would not prevent companies submitting data from including previously-generated LD50 data. If such data were to be included it would be of value in defining the toxicological profile of the chemical concerned. However, the Department's position is that it does not need LD50 data.[34]

7.75 Recently, regulations have been made in Victoria under the Prevention of Cruelty to Animals Act 1986. Regulation 24 deals with the Draize Test and Lethal Dose Testing and reads as follows:

(1) A person must not carry out any scientific procedure or series of related scientific procedures known as the Draize test to determine the relative irritancy of a chemical or a cosmetic, toilet, household or industrial preparation, using the conjunctival sac of rabbits.

(2) A person must not carry out any scientific procedure or series of related scientific procedures involving lethal dose testing unless:

(a) the scientific procedure is related to potentially lifesaving treatment or research in connection with cancer in human beings; and
(b) the objective of the scientific procedure cannot be achieved by any other scientific means; and
(c) the scientific procedure is recommended for approval by a Peer Review Committee established under section 34 of the Act; and
(d) the scientific procedure is approved by the Minister; and
(e) the scientific procedure is carried out in accordance with any conditions determined by the Minister.

(3) For the purpose of this Regulation, 'lethal dose testing' is any test for determining the relative toxicity of a chemical or a cosmetic, toilet, household or industrial preparation in which the object of the test is to assess the toxicity of the preparation against a predetermined level of mortality.

7.76 This regulation has however been subject to criticism. AFWA submitted:

> The banning of the LD50 test in Victoria has already slowed research in that State and will make it more difficult for the proposed Centre for Toxicology to operate effectively in Victoria. Experience in the Department of Pharmacology at Monash University indicates that it will lead to increased usage of animals rather than to a decrease.[35]

7.77 The Minister for Local Government and Planning in New South Wales announced in November 1988 his intention to propose amendments to the Chemical Research Act to restrict the conduct of certain tests in particular the Draize and LD50 tests. Applications to conduct such tests would be referred to the Animal Research Review Panel for review and would be subject to ministerial decision.

7.78 CSIRO has also called for a review of the need for this test.[36]

7.79 On 11 October 1988 the United States FDA stated of its current policy on the LD50 test:

> The statement provides a short history of FDA's policy on the "classical" LD50, including the fact that the agency revoked all regulatory requirements for the "classical" test in 1985. However, FDA "may not refuse to accept or review data, including acute toxicity data from the 'classical' LD50 test, if they are relevant to a decision FDA must make on the safety of a regulated article ... Thus, FDA cannot revise guideline test protocols or regulations to state that it will never use or consider any 'classical' LD50 data in making safety determinations."
>
> The policy further states, "The scientific community agrees that the 'classical' LD50 test is not necessary for determining acute toxicity. In agreement, FDA has adopted the policy that the 'classical' LD50 test is not a required toxicity study. The agency supports efforts to eliminate continued conduct of the 'classical' LD50 test and to reduce the number of animals used in acute toxicity testing without sacrificing information necessary in the interest of human safety."
> (NABR Update, Vol.9, No.22, 18 October 1988, p.1)

7.80 The Draize test is banned in Victoria and is subject to ministerial approval in New South Wales. It has been criticised on its effectiveness and on animal welfare grounds. It is also a

test which has been little used in Australia. The Committee believes that the Draize test is undesirable and RECOMMENDS that the Draize test be banned in Australia.

7.81 The Committee RECOMMENDS a ban on the classical LD50 test in Australia but that acute toxicity tests be allowed with ministerial approval. The classical LD50 is no longer required for registration purposes and is subject to ministerial approval.

7.82 For registration purposes, data derived from Draize tests or LD50 tests done overseas should still be accepted, provided that the relevant authorities and their advisers are satisfied that the data are valid.