anzccart HUMANE SCIENCE News

June 1998 Vol 11 No 2

How many animals?

The Australian Code of Practice for the Care and Use of Animals for Scientific Purposes states that proposals to Animal Ethics Committees (AECs) must justify the number of animals required, and that this should be "the appropriate number of animals (neither too few nor too many)" (NHMRC, 1997). No specific guidance is given as to how this number should be determined. The aim of this article is to bring to the attention of AECs and investigators objective statistical methods for determining required animal numbers. While these methods may incorporate information from the experience and judgement of the investigators, this is done in a defined and transparent manner, so that all aspects of the process may be subjected to the scrutiny of the AEC.

The use of sequential sampling techniques has been discussed in a previous issue of *ANZCCART News* (Chamove, 1996). One of the problems that AECs must grapple with when dealing with sequential designs is that the number of animals to be used cannot be specified in advance. The methods described here allow the required number of animals to be calculated before the experiment is commenced.

Different methods of determining animal numbers are required for different experimental designs. In complex situations, such as experiments with multiple treatment groups or multiple, possibly interacting, treatments, the advice of a statistician should be sought during the planning stages. In simple cases, for example involving the comparison of two groups of equal size, approximate determination of the required numbers is relatively straightforward and may often be undertaken by the investigator. Two cases will be described here. The first is for continuous outcomes, in which the response of each animal can take one of many possible values, and it is desired to determine whether the outcome is affected by an experimental treatment. Blood pressure, serum enzyme concentrations and body weight are examples of continuous outcomes. In the second case, the outcome is dichotomous, i.e. some characteristic is either present or absent, and it is desired to determine whether the proportion of animals with the characteristic is affected by the treatment. Examples of dichotomous outcomes are cured vs not cured, infected vs not infected. Sometimes it is useful to convert a continuous outcome to a dichotomous outcome, e.g. blood pressure elevated vs not elevated.

1. Continuous outcomes: Student's 2-sample t-test

A common experimental design involving a continuous outcome compares the mean response of a control group of animals to the mean response of a treated group. If the data are to be analysed by Student's 2-sample t-test, the approximate required sample size can easily be determined. The investigator must specify the desired Type I error, the desired Type II error, the minimum treatment effect to be detected, and the variability of the data.

Type I error

The Type I error, or α, is the highest significance level which will be accepted as demonstrating a difference between treatments. This is commonly set at 0.05. A lower level, such as 0.01, might be preferred if the hypothesis to be tested is especially novel. A smaller Type 1 error will require more animals.

From a table of the standard normal distribution, a value Z_α is determined as the value which includes $(1 - \alpha)$ of the area under the normal curve. For a 2-tailed test with Type I error of 0.05, Z_α = 1.960, as 0.025 of the area falls above 1.960 and 0.025 falls below -1.960. For a 1-tailed test with a Type I error of 0.05, Z_α = 1.645, as 95% of the area falls below 1.645.

Type II error

The Type II error, or β, is the probability that the experiment will fail to detect a significant difference when there is, in fact, a true difference of the magnitude specified. The

Contents ...

This issue contains the Facts Sheet on the Importance of non-statistical design in refining animal experimentation. The Facts Sheet is also available as an offprint.

For another discussion of the use of statistical design to reduce animal use, see the paper by Arnold Chamove in *ANZCCART News*, September 1996, **9**(3): 6-8

Type II error is commonly set in the range 0.05 - 0.2. A smaller Type II error will require more animals. The power of the experiment is 100 x (1 - β)%, so an experiment with β = 0.1 has a power of 90%. From a table of the standard normal distribution, Z_β is determined as the value below which (1 - β) of the area falls. For a Type II error of 0.1, $Z_\beta = 1.282$.

Minimum difference to be detected

Obviously if the treatment effect is very small, a large number of animals will be required to detect it. But a very small treatment effect may be of no importance. The investigator must specify the smallest difference in mean responses of the two groups, Δ, which is worth detecting. The smaller Δ, the more animals will be required.

Variability of responses

In order to determine the required sample size, the standard deviation, σ, of the outcome measure is required. In general, this is not known, and must be estimated. Ideally, the estimate would be based on a large number of measurements taken in animals under conditions similar to those of the proposed experiment. Sometimes the investigator may be able to make an estimate of σ from a knowledge of the response being measured. Usually it is easier to estimate the range. If the expected range in a sample of 100 observations can be estimated, this range divided by 5 can be used as an estimate of σ. Sometimes a pilot experiment may be required to estimate σ. The larger σ is, the more animals will be required.

Calculation of sample size

The number of animals required in each treatment group is calculated by Equation one (Guenther, 1981): Some commonly required values of $(Z_\alpha + Z_\beta)^2$ are given in Table 1.

$$n = \frac{2\sigma^2 (Z_\alpha + Z_\beta)^2}{\Delta^2} + \frac{Z_\alpha^2}{4}$$

Example

Gill (1978) describes an experiment to determine whether rates of weight gain of steer calves fed a new experimental ration differ from those receiving a control ration. The experiment should be sensitive enough to yield a significant result if the true difference in rates of gain is 0.1 kg/day (Δ = 0.1). It is required that the Type I error be not more than 0.05 and the Type II error not more than 0.2. Table 1 gives the required value of $(Z_\alpha + Z_\beta)^2$ as 7.9. Previous data indicate that the standard deviation of weight gain (σ) is 0.083 kg/day. The number of animals needed in each group is given by equation 1 as:

$$n = 2(0.083)^2 (7.9) / (0.1)^2 + (1.96)^2/4 = 11.8$$

It is usual to round the result up to the next whole number to ensure that the Type II error will not exceed the value specified. Twelve animals per group will therefore provide the required power for this experiment.

2. Dichotomous outcomes: Chi square test

Where the outcome is dichotomous and results will be analysed by a chi square test, in addition to the desired Type I and Type II errors, the investigator must specify P_C, the proportion of control animals expected to show the response, and P_T, the proportion of treated animals responding which is required to give rise to a significant result. The number of animals required in each group is given by Equation 2 (Fleiss, 1981):

$$n = \frac{[Z\alpha\{2P(1-P)\}^{1/2} + Z\beta\{Pc(1-Pc) + P_T(1-P_T)\}^{1/2}]^2}{(P_C - P_T)^2}$$

where $P = (P_C + P_T)/2$

Because the raw data are counts, the test statistic computed in the chi square test can only take certain discrete values. The Yates' continuity correction is a modification to the chi square test which is intended to improve the fit of the test statistic to the continuous chi square distribution if the null hypothesis is true. While statisticians argue over the appropriateness of using Yates' correction (Haviland, 1990), it is recommended by many statistics textbooks and commonly used in the biomedical literature. Use of Yates' correction results in reduced power, and consequently a requirement for more animals, than use of the uncorrected chi square test. The required sample size can be determined by computing n using Equation 2, and then computing the adjusted sample size, n', by Equation 3:

$$n' = \frac{n}{4}\left[1 + \left(1 + \frac{4}{n|P_C - P_T|}\right)^{1/2}\right]^2$$

where $|P_C - P_T|$ is the absolute magnitude of the differences in response. Results based on Equations 2 and 3 have been tabulated by Fleiss (1981).

Example

Snedecor and Cochran (1980) give an example in which a standard antibiotic protects 50% ($P_C = 0.5$) of animals against a disease. An experiment is to be carried out to determine whether a new antibiotic is superior. A power of 90% is required (β =

TABLE 1

Some values of $(Z\alpha + Z\beta)^2$

α	β	$(Z\alpha + Z\beta)^2$	
		1-tailed test	2-tailed test
0.05	0.05	10.8	13.0
0.05	0.1	8.6	10.5
0.05	0.2	6.2	7.9
0.01	0.05	15.8	17.8
0.01	0.1	13.0	14.9
0.01	0.2	10.0	11.7

0.1, $Z_\beta = 1.282$) with a Type I error of 0.05 to detect a difference if the new antibiotic protects 80% ($P_T = 0.8$) of animals. In this case a 1-tailed test is appropriate as interest lies only in whether the new antibiotic is superior. The required value of Z_α is therefore 1.645.

Calculating P = (0.8 + 0.5)/2 = 0.65, we use Equation 2 to determine n:

$$n = [1.645 \{2 \times 0.65 \times 0.35\}^{1/2} + 1.282 \{0.5 \times 0.5 + 0.8 \times 0.2\}^{1/2}]^2 / 0.3^2 = 41.4$$

Snedecor and Cochran (1980) arrive at a group size of 40, using an equation which is simpler but less accurate than Equation 2.

If it is intended to use Yates' continuity correction, the required sample size will be:

$$n' = \frac{41.4}{4}[1 + \{1+4/(41.4 \times 0.3)\}^{1/2}]^2 = 47.8$$

Therefore, 48 animals per group will provide the required power.

3. More complex cases

Appropriate sample size may also be determined where it is desired to use unequal group sizes (Machin *et al.*, 1997), more than two treatment groups (Gill, 1978), or where nonparametric methods of analysis will be used (Lehmann, 1975; Machin *et al.*, 1997). It is recommended that a statistician be consulted during the planning phase of complex experiments.

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1998 NEAMS Grant awarded

The New Educational Aids in Medicine and Science (NEAMS) Trust has awarded $10,000 to Dr Rodney Coster, Dr Peter Sylaidis, Dr Timothy Proudman and Ms Elizabeth Owen of the University of Adelaide's Department of Surgery, based at the Queen Elizabeth Hospital, Adelaide.

The grant will be used to develop an interactive CD-ROM dealing with basic surgical skin wound management for medical and dental students and for general practitioners. Benefits could extend to veterinary and biomedical students. The CD-ROM will guide users through a range of options of skin wound management, including anaesthesia, suturing and wound care.

One of the main outcomes of the project will be to reduce the need for animal models. Currently these techniques are taught using abattoir material and experimental animals.

For further information about the NEAMS Trust or this grant, contact Associate Professor Garry Scroop, Department of Physiology, University of Adelaide.

For further information about refining animal experiments, see the Facts Sheet in this issue, by Professor David Morton

Animal replacement and reduction: multimedia teaching aids in behavioural pharmacology

Pharmacology is traditionally a high user of animal tissues in undergraduate practical class teaching. To some extent, much of this animal usage is tied to the number of students entering the course. Within the Department of Pharmacology, student numbers have progressively increased; with an increase of approximately 500% in student practical class numbers over the past five years.

Clearly, a similar, concomitant increase in animal usage is untenable; yet it is also important to maintain the aims of the practical course in reinforcing and illustrating basic pharmacological concepts, understanding experimental design and learning good laboratory practice by recording, analysing and extrapolating from results obtained in pharmacological experiments. A goal of the Department has been to reduce animal usage while maintaining teaching excellence by the introduction of multimedia approaches to teaching in applicable practical classes. The Department currently runs three types of practical classes:

- *in vivo* only experiments measuring behavioural responses of conscious animals and/or physiological parameters such as blood pressure or cardiovascular reflexes in conscious or anaesthetised animals;
- *in vitro* only experiments, using isolated animal tissues; or,
- a combination of *in vivo* and *in vitro* experiments.

As a first step towards animal usage reduction, we have developed a computer based multimedia approach to target classes that involve *in vivo* experiments measuring behavioural responses. Prior to the introduction of such an approach, students in these classes carried out a behavioural screen where the actions of drugs were observed in whole animals. Various behaviours were observed and scored giving a "behavioural map" for the effects of the drug in terms of its effects on:

- awareness and mood;
- motor activity;
- posture, coordination and muscle tone;
- reflexes; and
- the peripheral autonomic nervous system.

Students were expected to establish a profile of the behavioural effects of families of drugs; understanding the major effects of these drugs on the central nervous system and behaviour, as well as noting the similarities and differences between families of these drugs. The problems with using animals in the behaviour screen practical classes were:

- increased animal usage with increased class sizes;
- limitation on the types of drugs and behaviours examined (for example, it is not ethically tenable to evaluate the effects of potent central nervous system drugs such as convulsant drugs in a limited undergraduate practical class); and
- difficulty in reproducing "typical" responses in a small experimental population.

In 1996 and 1997, Dr Darren Williams developed the *Behavioural Pharmacology on CD-ROM* package with assistance of Dr Roger Summers and myself. The development of this package and its computer-based multimedia approach has addressed most of the problems with our animal behaviour practical classes.

At its simplest level the package:

- allows the student to review a larger range of drug families. Students can review several drug families including hypnotics and sedatives, neuroleptics, cholinergics, convulsants, opioids and central stimulants;
- provides additional text-based material on the basic pharmacological properties of the drug family and on key members of that family (figure 1);
- reviews the types and uses of behavioural tests. This is illustrated as short video clips of behaviours seen in a behavioural screen;
- reviews the effects of various drugs (figure 2); and
- provides for the student a series of self assessment tests on the drugs in context with the behavioural profile where students can review their understanding of a behavioural screen. In most cases, questions are coupled to a video clip that further tests their knowledge of behaviours seen in response to a variety of drugs.

All this is presented to the student with figures, illustrating drug structures and "live-action" video clips illustrating the behavioural responses themselves and the effects of drugs on these behavioural responses. Much of the material is "hot-keyed" and linked to various other parts of the material presented in the program, allowing students to follow various paths in tackling aspects of a behavioural screen, while at the same time giving students an opportunity to review material with which they are less familiar.

Generally speaking, the use of a computer-based multimedia approach offers many advantages. In our Department the use of the *Behavioural Pharmacology on CD-ROM* package has certainly decreased animal usage in a specific practical class and similar approaches would be of great benefit in minimising animal usage in many other practical classes.

Furthermore, the scope of the practical class has been broadened; additional material can be incorporated which may be impractical, time-consuming or too difficult to present to students in a practical class setting under normal circumstances.

This brings me to what I see as some of the limitations

of such an approach. Students will always see "ideal" responses; however, they will not appreciate the nature and form of the normal biological variability seen in any animal's response to a given drug.

To some extent students are removed from participating in an experiment and are less involved in decision-making that is important in learning processes. These factors, however, are being overcome with approaches that are more interactive, allowing students to actively participate in the presented experiment.

In conclusion, the use of computer-based multimedia is an extremely valuable tool to present a large range of material in different formats to pharmacology students while minimising animal usage in practical classes.

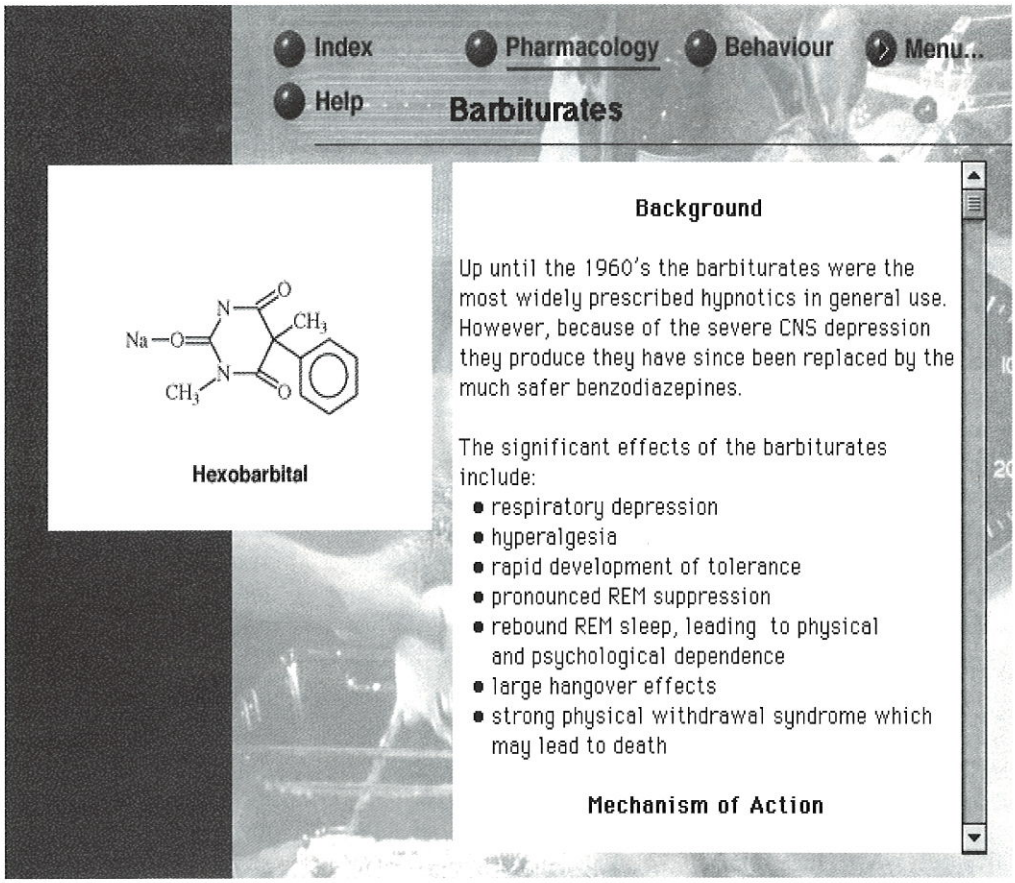

Figure 1
Within the major drug classes individual drugs can be reviewed. This figure shows some pharmacological properties of hexobarbital as an example of the barbiturate family.

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Editor's Note:
See page 8 of this issue for a review of a newly published list of alternatives to the use of animals for teaching undergraduate students in biological science, veterinary and human medicine.

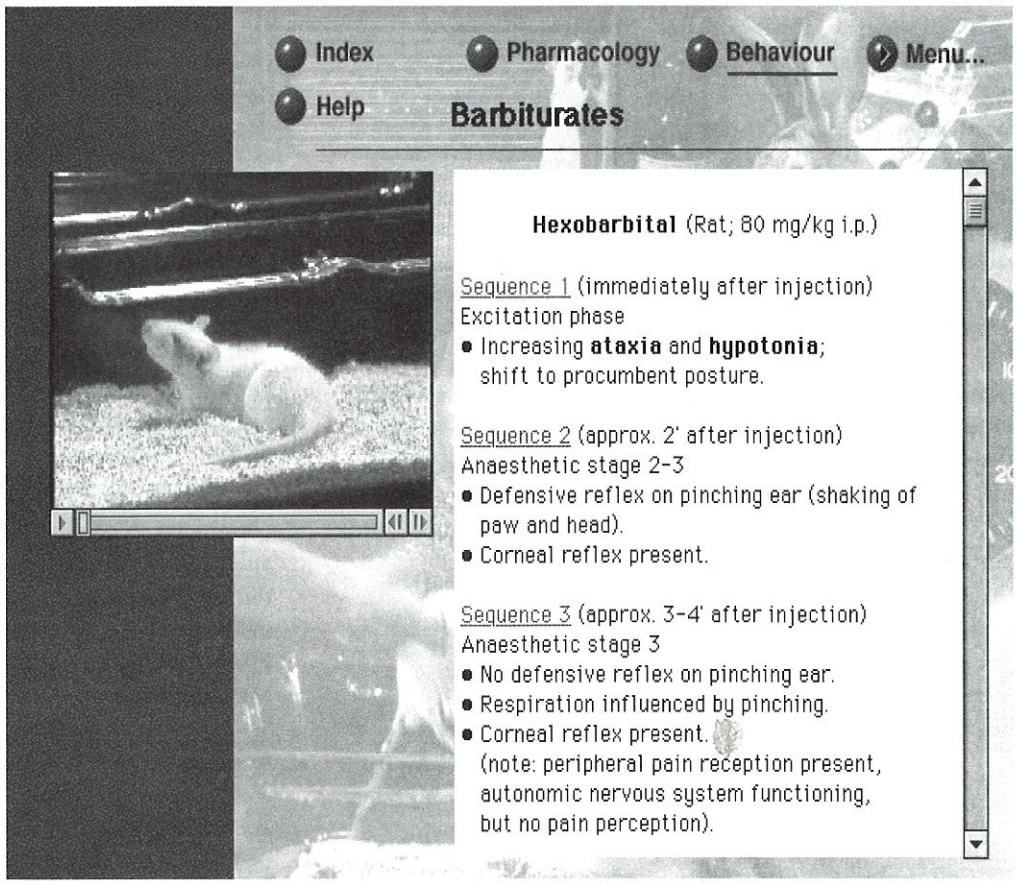

Figure 2
The effects of specific drugs are shown in several video-clips; in this example the time course of the effects of hexobarbital in a mouse can be observed. Text-based material provides further information of key aspects of the animal's response to the drug.

The current legal status of animal experimentation in Israel

In 1994, the Israeli Parliament promulgated a revised statute pertaining to animal experimentation. Accordingly, all animal experiments now need approval. As accepted in many developed countries, animal experimentation must be humane, i.e., with minimal pain and suffering to the experimental animal. Animal experimentation is permitted in order to advance knowledge in science and medicine, for diagnostic purposes and for teaching. Animal experimentation is not allowed when the experiment is deemed as being non-contributory to the advancement of knowledge, diagnosis or teaching, such as the testing of cosmetics or cleaning materials. In this article, a description of the application of this law is presented with attention given to the composition and responsibilities of various administrative bodies and individuals empowered to ensure its enforcement.

National Council for Supervision of Animal Experimentation

The National Council for Supervision of Animal Experimentation is the legally constituted body responsible for the enforcement of the law pertaining to animal experimentation.

Composition

The committee comprises 23 individuals, each representing different organizations with an interest in animal experimentation, and whose appointment is subject to the approval of the Minister of Health. The term of office of each committee member is four years. The 23 committee members are:

- six members from the National Academy of Sciences with two persons representing life and/or medical sciences and one person representing the social sciences, the humanities, the exact sciences and jurisprudence.
- the Director of the Veterinary School or his/her representative;
- deans from two of the medical schools or their representatives;
- a person representing the Israel Veterinary Medical Association;
- a veterinarian appointed by the Scientific Committee of the Israel Veterinary Medical Association;
- six individuals representing the Israeli Ministries of Health, Education, Science, Defence, Justice, Religious Affairs and Environment;
- a person representing industry;
- the Director-General of Veterinary Services, Ministry of Agriculture or his / her representative; and
- three representatives from Animal Welfare organizations.

Responsibilities

The responsibilities of the committee are:

- to establish guidelines for animal experiments specifically targeting the areas of minimizing the suffering of experimental animals and to prevent unnecessary or redundant experiments;
- to establish syllabi of courses suitable for teaching the practical aspects of humane animal experimentation to researchers, students and auxiliary staff;
- to license institutional animal care and holding facilities with respect to its physical structure and to ensure that the housing of experimental animals and the practice of laboratory animal husbandry satisfy internationally accepted standards;
- to survey the approvals granted by approved institutional Animal Ethics Committees;
- to review applications for permission to conduct an animal experiment where the establishment of an institutional Animal Ethics Committee has not been approved;
- to represent the scientific community on matters and issues related to animal experimentation to the general public;
- to educate the general public on all aspects of animal experimentation.

A veterinarian appointed by the National Committee is responsible for its supervisory activities.

Animal Care Facilities

Any institution that conducts animal experiments must have a licensed animal care facility. The requirements for licensing are determined by the appropriateness of housing of each animal species and the relevant animal husbandry practices, similar to those outlined in the *Guide for the Care and Use of Laboratory Animals* National Research Council, National Academy Press, Washington DC, USA, 1996.

Each institution with a licensed animal care facility must employ a veterinarian with specialist training in laboratory animal medicine and husbandry.

The responsibilities of the veterinarian are:

- management of the facility;
- the health and welfare of its residents;
- advising approved animal experimenters on the health and welfare of their experimental animals;
- conducting courses in the practical aspects of animal experimentation, such as animal handling and restraint, methods of anesthesia, analgesia, and euthanasia, to ensure that individuals conduct their

The importance of non-statistical design in refining animal experiments

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ANZCCART Facts Sheet

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Introduction

Refinement, one of the Three Rs, is perhaps the area where most progress can be made to reduce any animal suffering incurred in animal experimentation in the short term. Another 'R', Reduction, will reduce the number of animals used in research through good statistical experimental methods and design. This paper shows how non-statistical strategies can contribute markedly to minimising pain and suffering in an experiment. The third 'R', the Replacement of animals in experiments, however, is a longer term aim which requires considerable investment, and input of basic animal biology data, before absolute animal alternatives can be made available to provide reliable scientific data. In the past 20 years or so, Replacement has developed as a concept more in relation to toxicity testing and finding ways in which the necessary harms done to animals (e.g., the necessity to produce clinical signs of toxicity in order to determine the toxicity of a chemical) can be eliminated. In fact, less than 10% of animal experiments involve toxicity testing (UK Home Office Statistics, 1996). The use of replacement alternatives in the other 90% of experiments is less likely as whole animals are needed. This is because the purpose of the experiment is not simply to determine toxicity but to determine function of part of the body (eg. the immune system, the reproduction cycle) or to test the effectiveness of new medicines (eg. antibiotics, anti-hypertensives, anxiolytics, analgesics, anesthetics) and so require whole animal body systems to be fully functional. Nevertheless, even in these situations, *in vitro* methodology can provide important information for the investigator. It may seek to answer questions such as whether a chemical interacts with cell receptors, or at what level is it toxic, or does it fail to interact?

Refinement

Refinement not only attempts to reduce negative states in animals, but promotes positive mental and physical states. It can be defined as follows:

> *Those methods which avoid, alleviate or minimise the potential pain, distress or other adverse effects suffered by the animals involved, or which enhance animal wellbeing.*

The scientific paradigm is normally to vary one parameter at a time in some form of standard experimental protocol. Thus identifiable variables are limited as much as possible in any one experiment, so that only the specific variable of interest is examined. This objective will be compromised not only by poor experimental design but also through inadvertent side effects which can occur as a result of compromised animal wellbeing. Unintended animal suffering deriving from physical damage to an animal, or physiological or psychological perturbances from normal, can materially affect the scientific data collected and so should be avoided as far as possible. Avoiding such confounding effects is more than simply the provision of anesthetics and analgesics when animals may be in pain, or alleviating distress and fear. While these are very important, there are *additional* strategies which can be employed to reduce the level of animal suffering, notably the statistical and non-statistical design of experiments. Traditionally, research into the refinement of experiments has not been rigorously investigated as it is not seen to be part of the main line of research. Consequently, important questions such as how much pain should be caused to demonstrate effective analgesia are often ignored. If scientists wish to claim they practise humane science then they have to pay as much attention to avoiding unnecessary pain and distress to their animals as they do to their scientific objectives.

Humane science will influence the welfare of the animals as well as the scientific quality of the work, and deserves detailed consideration. Moreover, attention to refinement will have a beneficial effect on the costs of the research, because both experimental design and the welfare of the animals affect the variance in the scientific results. Poorly designed experiments will be likely to use more animals than necessary to achieve the scientific objective, which in turn will lead to increased costs for upkeep, administration, record keeping and treatment. As important scientific discoveries often have to be validated by replication of the work in another laboratory, this too will add to the overall costs if the work has been poorly carried out, especially if not accurately and fully reported. It is important that scientific protocols, including the precise experimental design, are recorded in detail in peer reviewed journals (Morton, 1992). Factors leading to good animal welfare will usually also lead to reliable, accurate and economic science.

Literature review and choice of model

There are three primary points to establish before embarking on any experiment. Has the work been done before; is there an alternative way of achieving the scientific objective without the use of sentient animals (eg. using invertebrates, or *in vitro* techniques); and is the scientific question worth answering, in the light of the pain and suffering that may be caused to the animals during both their husbandry and the experimental procedures.

It is also important to review the literature *before* starting any work, focusing not only on whether the proposed approach to the scientific objective is appropriate, but carrying out a critical analysis on whether the proposed model really will achieve the stated objectives. If there is a choice of models then the advantages and limitations of each should be considered. For example, is the model simply reproducing the clinical signs of a disease, or reflecting the actual cause of the disease, such as the infective agent or the genetic defect (it is in this area that transgenic animals should be improving the validity of certain animal models of human diseases)? Has the model been validated in any way, if so how, and is it scientifically acceptable?

The choice of species can be critical when the results are to be extrapolated to humans. Those animal species with similar genetic predispositions, or metabolic pathways, or that show similar pathology to humans are likely to be better models. When the purpose of the work is specifically aimed at a non-human species (eg. veterinary, zoological or wildlife research) then other considerations come in, such as the stresses involved in capture or confinement. It is also important to remember that there are strain or breed differences within a species which can be critical (Claassen, 1994; Hendriksen and van der Gunn, 1995). Both species and strain should be comparable for scientific validity.

Other questions include whether adequate facilities are available locally to create the model. Is the necessary expertise available to recreate it accurately and precisely; and are the staff able to care for the animal afterwards and during its lifetime (Smith and Boyd, 1991)?

In some countries special justification is required for the use of some species, eg. in the UK the use of dogs, cats, horses and primates. This is not related to the biological needs of these animals, or to their ability to feel pain more than other species, rather it is an attempt to reflect the special public concern over the use of some species compared with others. Those species that are often eaten, regarded as pests, or are at least distasteful in some way or another, even though they may also be kept as pets (eg. mice, rats, gerbils, hamsters, guinea-pigs, rabbits) cause least public concern. Surely, the rational choice of species is the one that is the least sentient consistent with sound science.

The use of anaesthetics and analgesics

Scientific procedures should be performed under anesthesia (ie. general, regional, local or with analgesia) unless either the pain or discomfort of the procedure is less than that caused by the anaesthetic; or its use would be incompatible with the scientific objectives. The latter has to be shown and not assumed and a scientific approach is the best way to do this. Often assumptions are made to the detriment of animal well-being, and possibly also to the quality of the science, because of the animals' responses to stressors; but by not researching this, it is never revealed. In addition the type of anaesthesia should be suitable for both the species and the duration of the procedure and the researchers should be competent in its administration. If no anaesthesia is to be used it is important that pain and fear should be kept to a minimum through the use of analgesics and tranquillisers.

The importance of competent and skilled investigators

Competent and skillful researchers are vital for the production of good science. Poorly carried out techniques can cause considerable, and avoidable, suffering and it is in the interests of both science and animals that all scientific procedures are carried out in the most humane manner. A new technique that demands new skills should be carefully learned by an investigator before embarking on an experiment in which animals recover and data are recorded, even if more animals have to be used. Becoming competent in a technique involves watching those who are competent and learning the 'tricks of the trade'. Depending on the technique to be learned, it may be appropriate to dissect dead animals to learn the anatomy and to understand the inherent dangers of inaccuracy. The equipment to be used must be suitable to the species, strain, sex and size or age of the animals. For a surgical technique, the next stage may be to practise on recently killed animals so that the tissues are warm as this can markedly alter their texture and hence the value of the learning experience for the operator. Finally, a surgical technique can then be applied to anaesthetised animals which may be allowed to recover if it has gone well, but if not, those animals should be killed. The importance of learning with a competent, skilled and up-to-date scientist or technician cannot be overemphasised. Moreover, even simple techniques on conscious animals like handling, oral dosing, parenteral administration of substances and removal of blood, if carried out badly, can significantly elevate the level of animal pain and distress. This in turn will cause further problems next time as the animal anticipates what is about to happen based on its previous experiences.

Statistical advice

It is well worth obtaining statistical advice *before* an experiment is started. There are many standard texts and commentaries (eg. Mead, 1988; Erb, 1990; Mann et al., 1991; van Zutphen *et al.*, 1993; Festing, 1994; Erb, 1996; Chamove, 1996; Khamis, 1997) covering the various approaches. They include concepts such as

avoiding using too few animals during an experiment as opposed to too many; what data are to be collected and how the data should be analysed; the value of pilot studies and prior information of expected variance; the calculation of the number of groups, the number of animals in each group and increasing the power of an experiment through blocking; ongoing analysis during an experiment; as well as statistical instruments and tests.

The limitations of scientific evidence, extrapolation and animal numbers

The purpose of an experiment has to be clearly defined, including the primary application of the results. For instance, in safety testing for a new chemical entity, the necessity to obtain acute toxicity data to a probability of <0.001 is pointless when an extrapolation is to be made to another species, normally the human. Moreover, if a fudge factor, such as a further safety margin of 100x the toxic dose found in animals, is going to be used to calculate the hazard involved in any exposure for humans, say for the transport of that substance, then the futility of such precise experimentation is highlighted even further. The question to be asked is, What is going to be done with the results? When there is a clear difference in consequent actions based on the difference in probability estimates, and the preferred action can be justified, the required animal use is acceptable. But if there is to be no difference in action between different statistical precisions then the lowest acceptable precision which will lead to the use of the fewest animals should be used.

Another instance is when it has been decided that even if one animal in a group fails a test, then a specific action will take place. For example, if a substance is going to labelled as toxic on the basis of one animal in a group of six reacting adversely, and if the first animal reacts adversely, then there is no point in continuing with the experiment and giving that substance to the remaining five animals. It would have provided valid scientific information, of course, but the practical applied outcome has already been decided and so there is no point in continuing the study and causing pain and suffering to the remaining animals.

Protocols evaluating new treatments, eg. the rodent protection test (RPT) help determine, for example, effectiveness and therapeutic levels of antibiotics for use in humans. In the RPT, the effectiveness of a novel compound to prevent or treat infection is investigated over a range of doses using standard micro-organisms in an animal model (mouse or rat). If, compared with other similar drugs, the compound appears to have advantages (eg. showing a better bacterial kill rate or less toxicity) then the information gained from the animal studies will be taken into account when Phase one (healthy human volunteers) and Phase two (human patients who can expect to benefit therapeutically and in whom effective dose levels will be determined) studies are undertaken. There is little need for high precision in the animal model species as opposed to the target species and yet the approach of accepting a lower statistical probability ($p < 0.05$ cf. $p < 0.01$) may mean the difference between 5 and 50 animals in a group. This is especially important in the area of infection where considerable suffering may occur in control animals which are not protected from the disease.

Pilot studies, dose sighting, and control groups

Pilot studies can be useful to give some idea of the variance, the likely adverse effects that may be encountered, and any practical local problems that are likely to be encountered during the proper study. They help to determine the appropriate number of animals that should be used in any one group, as well as the avoidance and alleviation of any adverse effects. The use of pilot studies need not be a waste of animals, as the results can be used for the main study, unless there are good reasons for not so doing.

Historical data in this regard are also very useful, particularly when considering both positive (eg. a standard challenge of a known substance) and negative (eg. giving vehicle alone) controls. This use of background data can influence the number of animals needed for a given study. In some instances it may not even be necessary to have a control group as the control 'fact' is so well known and accepted eg. total pancreatectomy leads to diabetes mellitus with a consequential rise on blood sugar and death in less than 10 days. However, it may be advisable to carry out pilot studies when using a new model in one's own animal facility as differences in strain of animal, diet, staff, bedding, cleaning materials, husbandry and environment have all been shown to affect animals' responses in various ways (see Claassen, 1994; Morton, 1995; Wadham, 1996). This was exemplified by a multicentre trial on the Fixed Dose Procedure carried out by van den Heuvel and co-workers (1990). They found that while the LD50% of various chemicals were similarly ranked between laboratories, the actual LD50% dose varied considerably.

There are several methods for determining the relevant dose(s) for animals *in your facilities* in pilot studies but the up and down method deserves special mention (Bruce, 1985). In this approach, a single animal is given a dose (eg. from the literature) and its response noted. Assuming that the first dose does not produce the anticipated response, a second animal is given a higher or lower dose. This is then repeated on individual animals at varying dose intervals until a suitable dose rate is found. This approach is helpful for streptozotocin dosing to induce diabetes in various strains of rats, as well as for other chemicals given to induce various disease states.

Advice from other scientists, veterinarians and caretakers on adverse effects

The first time a novel scientific procedure is carried out will require a prediction of the type and incidence of any adverse effects on the animals, but thereafter the experience gained will be helpful in future work using that or similar protocols. While a thorough review of

the literature is essential, sadly the adverse effects on the animals encountered are rarely published in a helpful manner for those using the model (see Morton, 1992). Furthermore, the 'tricks of the trade' so often vital to be able to repeat the work are omitted, let alone relevant details relating to the animal side of the work, e.g., failed approaches, details of animals used as well as the husbandry system. The use of score sheets (Morton, 1994, 1995, 1998b; Townsend and Morton, 1994; Morton and Townsend, 1995) help all those involved in the research program to recognise the cardinal clinical signs that animals show during a specific scientific procedure, and can signal subsequent actions, notifications and treatments to be taken when certain clinical signs are observed (eg. humane end points). Animal carers, stockpersons and veterinarians who are familiar with the animals in the experiment are excellent guides in determining any adverse effects. They may not always be able to explain why an animal is 'wrong' or 'not right' but good ones are rarely mistaken! The score sheets start to explain their insight and simple tests like giving an analgesic and observing if animals change their behaviour such as by moving around more, eating and drinking, all add empirical evidence to suggest those animals were affected by that protocol and suffering in some way (see below for more details).

In vitro to in vivo

Research is sometimes aimed at investigating the interactions of substances, such as new drugs, in the whole body. If the *in vitro* work will yield information that shows the effectiveness of the substance, or viability of the cells/organisms has been compromised in some way, it may not be worth proceeding with the *in vivo* work. There has to be confidence that the *in vitro* work is able to be extrapolated to the *in vivo* situation, and if so the *in vitro* work should precede the *in vivo* experiments. For example, genetic modification of viruses may reduce their virulence so that they are no longer able to infect cells *in vitro* and, based on past experience, it is unlikely they will infect cells *in vivo*. Similarly, radiolabelling of bacterial toxins may remove their toxicity, which can be measured by a change in binding characteristics to monoclonal antibodies, or to cell surfaces *in vitro*, or to a change in toxicity to less sentient animal forms such as invertebrates. Again, based on experience, it may be unlikely the modified toxin will be effective *in vivo* and so these experiments need not be done. In cases of doubt, however, small pilot projects involving only two or three animals can be used (depending on the precision needed) to confirm such findings and so validate a predicted negative, especially when there is less likelihood of causing animals pain and suffering. When a positive harmful effect needs to be confirmed *in vivo* then only one or two animals may be needed as *any toxicity* will be likely to provide sufficient evidence. For example, it may render a product such as a shampoo causing corneal ulceration or even corneal rupture unmarketable. Furthermore, in such cases where an adverse effect is predicted and needs to be confirmed, it may be possible to prevent the animal feeling any pain by carrying out the work under general or local anaesthesia.

Specific tests may be carried out to determine if an experiment is likely to work *in vivo*, or whether it is unlikely to succeed for some reason. If a chemical is cytopathic i.e., toxic to cells *in vitro* at concentrations that were predicted to be useful *in vivo*, then the work should not proceed. This is the basis of the so-called pre-screening tests used in the early stages of toxicity testing of new chemicals and medicines. An example of this might be a chemical in solution which at the desired concentration has a pH which is far from being biocompatible and will obviously cause tissue damage if given parenterally. It may then be given by some other route e.g., enterally, or at a lower concentration or in a different formulation. In all these cases consideration is given to carrying out *in vitro* work before whole animal work in order to avoid, or alleviate better, any adverse effects in animals.

Within in vivo work

Even within *in vivo* work it may be possible to stage work so that serious adverse effects are avoided. For example, if one is looking at the survival of animals given a bowel transplant, the animals are likely to die painfully from a peritonitis. Whilst one could wait for clinical signs of peritonitis to become apparent, it is also possible to avoid that altogether by trying to pick up signs of very early rejection, rather than bowel perforation (caused by rejection leading to death of intestinal wall cells and disintegration of the bowel). The early signs of transplant failure may be thrombosis of the vessels leading to the graft within a few hours of the end of surgery. By keeping the animal under a terminal anaesthetic for say 12 hr or so, one can monitor blood flow and the condition of the transplant (colour and contractility) and if found to be deleteriously affected, the animal can be killed before it recovers from the anaesthetic. If successful after 12 hr or so, the animal can either be permitted to recover, or I would recommend that the next animal be permitted to go for 24 hr before being killed. In such a way animal suffering can be reduced considerably, and it may be possible to avoid serious adverse effects altogether.

Another approach is always to do key pilot experiments before any controls or further experiments. Experiments should be designed to provide crucial information on whether it is worth progressing down certain experimental routes. For example, if the experiment is to determine the effect of a substance or specific cell or tissue on a physiological response, then a pilot study showing first that that response actually occurs should be undertaken. Only then should the main study and necessary controls, such as using saline or sham operations, be carried out.

Mild to high severity

Several examples can be instanced where the harms done to an animal can first be shown to be effective or ineffective at a low level before going to higher levels of animal pain and suffering. Traditionally this

aspect of refinement has not been rigorously investigated as it is not seen to be part of the main research, and the 'standard/traditional' time/level normally used. In some psychological experiments animals may be starved for long periods in order to make them 'work' in some way or another. If the animal can be motivated to work by reward rather than by starvation or even aversion stimuli, then such a strategic progression of harm could reduce the amount of animal suffering without losing the scientific objective. If starvation is used to motivate the animal then how long should it last – 48hr, 24hr, 18hr, 12hr or 6hr – and does it depend on the age of the animal, the species or strain? The adverse physiological effects of such periods of starvation (and even dehydration) on the animals have also to be considered and whether this will affect their performance and hence the science. If aversion has to be used, then the same principle should apply: use the lowest level of aversion (e.g., electric current, sound level, learned helplessness) necessary to achieve the scientific goal. Testing of novel analgesics, or determining neurocircuitary or transmitters may be possible through causing low levels of painful stimuli rather than high ones. An analgesic ineffective at low levels of pain is unlikely to be effective at higher levels of pain. The neurobiology may change quantitatively but not qualitatively at the higher levels of nociception. In any event, the purpose of the experiment has to be explicit, and justification made for the higher pain levels. Finally, the purpose of dose sighting studies mentioned earlier and practised by the toxicologists in safety testing are not routine in other areas of science. A scientist may start a dose response experiment at a range of dose levels only to find all the animals are unaffected, or worse still all the animals have died. Pilot studies are always worth doing in these sorts of cases.

Other examples include only affecting the use of one of a paired organ, that is important for an animal's quality of life, rather than two eg. legs in fracture or arthritis or musculoskeletal research; sense organs such as eyes and ears; transplanting a third kidney into an animal in the groin where it can easily be monitored rather than removing an animal's own kidneys and replacing it with a donor kidney (this could also be an example of a staged approach if true dependence needed to be established). A similar approach can be adopted in the study of skin transplantation or burns research where a minimum area should be transplanted or damaged or to neural studies where nerve section or denervation is required: rather than incapacitating the whole leg through high nerve section, a low nerve section which will affect just one muscle in the distal part of the limb so that compensation is possible through the use of other muscles, should be carried out. In all these examples if the purpose is to study quantitative aspects then this can be staged so that small harms are first seen to be 'effective' in whatever scientific sense is appropriate, and then the insult can be scaled up providing the scientific justification is ethically acceptable.

Cancer research

A set of excellent guidelines exist for the refinement of experiments involving cancer (UKCCCR, 1997). The recent increase in cancer experimentation as a result of gene therapy work makes this an important area for refinement. In essence, the growth rate of tumors and their size should be kept to the minimum necessary to demonstrate effectiveness or ineffectiveness of novel therapeutic agents or regimes. Death can hardly ever be justified as an end point (Mellor and Morton, 1997). Tumors should be placed whenever possible in sites where they are easy to palpate or monitor. Animals should be monitored at least daily, and more frequently if necessary eg. at times when the tumor ulceration is likely to occur or the animal may be found dead. Other parameters indicating animal wellbeing should be sought as well as tumor size, particularly if the tumor is deep or invasive or internal and so difficult to detect. These may include the behavior of animals, their coat quality, posture and appearance, especially their behavior at night, a period when they should be maximally active.

Humane end points

It is often possible given sufficient familiarity and experience with an animal model or specific scientific procedure that the adverse effects on animals will be predictable. This will not only include the clinical signs an animal might show but also the duration of those signs, the progression to other signs, even death, the timing of such signs after a particular procedure has been carried out, and also the proportion of animals that may be affected. Actions can then be taken depending on the scientific purpose and also on humane considerations. Certain levels of harm may be integral to the procedure or unavoidable to achieve the scientific objective. However, if the animal, because of the deviation from normality either physiologically or psychologically, loses its scientific utility, then it should be humane killed as keeping it alive will yield no valid information. Similarly, if the scientific objective has been achieved, then there is no useful purpose in keeping the animal in pain or distress. If, however, it may yield useful information then it may be possible to alleviate the animal's condition and so reduce the level of suffering; or the level of suffering may have become incompatible with the expected degree of scientific benefit and on an ethical harm-benefit analysis, the animal should be killed. Alternatively, the animal may still have utility in a scientific sense but there was no need to have let the animal progress that far as earlier clinical signs could have predicted what was to come.

This idea of using early clinical signs to predict later ones requires validation studies where it is shown that animals will normally progress in that way and that such an end point can be relied upon. This may be important for safety studies in the testing of medicines such as vaccine potency testing, rodent protection test, virulence assessments, or batch testing of

natural or synthetic products. Cussler (1997) has recently presented some data on a lethal rabies vaccine potency test where he found that vaccinated mice given challenge doses of virus went through a series of predictable clinical signs. He showed that animals showing slow circular movements invariably progressed to death - the traditional end point for the test. Death, as so often is the case in many experiments, is not related to the experimental variable under study, but is due to indirect effects such as dehydration caused by animals not being able to drink and increased packed cell volume leading to heart failure. Another cause of death in mice can be low body temperature due to inadequate food intake. Sometimes death due to these indirect causes can take several days and Cussler in his work, and Soothill *et al.*, (1992) showed that animal suffering could be reduced by several hours to three to four days through implementing validated pre-lethal end points.

In the UK, under the *Animals (Scientific Procedures) Act* 1986, each scientific procedure has a severity limit which should not be exceeded, and indeed it is a criminal offence to do so. The question is how that limit is recognised in practical terms. In theory there are four recognised severity limits: mild, moderate, substantial and severe, but severe pain or severe distress is not permitted under any circumstance. In a sense, what the bands are called is irrelevant, what matters is how they are interpreted. In order to interpret them accurately and reproducibly, careful observation of animals is required, and score sheets documenting clinical signs with time are invaluable (Morton and Griffiths, 1985; Morton, 1985; 1986; 1990; 1994; 1995b; 1997a; 1998a; 1998b; Morton and Townsend, 1995). Severity limits can be interpreted as the degree of deviation from normality coupled with other indicators of health and quality of life. A body weight loss of up to 10, 20 or 25%, or greater than 25%, compared with controls may reflect the mild, moderate, substantial and severe limits. But animals with tumours or ascites may increase in body weight but lose body condition (ie. muscle mass) and be experiencing extreme suffering. Alternatively, animals with a body weight loss of 25% and which are diabetic or which have exocrine pancreatic deficiency, may be very lively and have a good quality of life. It is important that a holistic approach be taken so that clear clinical signs can be used to determine humane end points in accordance with the scientific benefit and humane research.

Score sheets - an approach to the recognition and assessment of animal suffering

Many laboratories are approaching the difficult topic of the recognition and assessment of suffering using score sheets ie. a list of cardinal clinical signs encountered in that particular scientific procedure. These are developed through experience and, by and large, are unique to the system of husbandry, to the specific experiment, as well as to the species, and even the breed or strain of animal, being used. It is not possible to make a general score sheet for all experiments and/or for all species. One only has to consider the different potential adverse effects of a skin transplant compared with a kidney or heart transplant to appreciate the different signs that might occur (eg. haemorrhage, rejection of skin compared with a dependent kidney or an accessory heterotopic heart in the abdomen). Lists of signs are developed by observing the first few animals undergoing a novel scientific procedure very closely and then the list is modified with experience until a set of cardinal signs that most animals will show during that experiment, and that are relevant to the assessment of suffering, is achieved.

These key clinical signs are set out against time in the score sheet (see Table 1). On the left hand side are listed clinical and behavioural signs and along the top the days and the time of the recorded observations. The method of scoring is that clinical signs can only be recorded as being present or absent indicated by a plus or a minus sign (or sometimes a +/- if the observer is unsure). The convention is that negative signs indicate normality or within the normal range, and positive signs indicate compromised animal wellbeing. In this way it is possible to visually scan a score sheet to gain an impression of animal wellbeing: the more plusses, the more an animal has deviated from normality with the inference that it is suffering more than it was earlier. Clinical treatments and other observations are also recorded. It is important to note that animals can be scored at any time and it would be certainly be more than once daily during critical periods when an animal's condition could predictably give rise to concern (e.g., in the immediate post-operative period; in a study on infection after the incubation period and at the time of bacteraemia or septicaemia).

Practically, it is important to develop a disciplined approach and strategy to the recognition of adverse effects in animals. At the beginning of an assessment the animal should be viewed from a distance, and its natural undisturbed behaviour and appearance are noted. Next, as the observer approaches the cage or pen the animal will inevitably start to take notice and interact with the observer and that interaction can be used to determine whether it is responding normally (an animal may become inquisitive or show signs of fear). Finally, a detailed clinical examination can be carried out by restraining the animal in some way and observing its appearance carefully and then making clinical measurements eg. of bodyweight and temperature, in addition to its behaviour as it may be more aggressive or fearful, or even vocalise.

At the bottom of the sheet there are guidance notes for the animal technicians about what they should provide in terms of husbandry and care for animals on that scientific procedure. There are also guidelines on how to score qualitative clinical signs such as diarrhoea and respiration, as well as criteria by which to judge humane end points. Finally, if an animal has to be killed there is guidance about what other actions should be taken, such as tissues to be retrieved and kept in formal saline to ensure that the maximum information is always obtained from any animal study.

While these sheets take time to fill in it is not diffi-

cult for an experienced person, such as an animal caretaker, to see if an animal is unwell, so the time taken can be reduced by simply scoring that the animal is normal by ticking the NAD box (Nothing Abnormal Detected). However, if an animal is not normal, it does take time to check it and to make judgements over what actions to be taken, but is that not the price for practising humane science?

In order to promote good care and good continuity of care an animal technician can be responsible for liaising with scientists and other technical staff, and also to maintain and update the score sheets. The roles of the technician in charge are:

- to check that the appropriate licences and permits are in order and correspond with what the scientist actually intends to do to the animal(s);
- to check the score sheet is appropriate before the experiment begins;
- to know the purpose of the experiment and the scientific objectives, and to become familiar with the scientific procedures to be carried out on the animals and the clinical signs that may occur;
- to ensure all personnel (technicians, scientists) know how to use score sheets and can recognise the clinical signs and can interpret the signs clearly into humane end points;
- to check that technicians not familiar with that experiment, say doing a weekend or holiday shift, are informed about the animals;
- to liaise with researchers over the experiment eg. timing, numbers of animals, equipment, end points;
- to update the score sheets based on new signs or combinations of signs observed; and
- to report to the responsible persons any concerns over the animals or personnel involved.

Table 2 shows a completed score sheet. At a glance it can be seen that there are more plusses to the right than to the left. Several other points can be noted: first, along the top, that as the animal became unwell, so it was scored more frequently. During Day 0 (the day of the operation) it scored abnormal in one or two predictable signs as it was recovering from the anaesthetic and the surgery (low body temperature and hunched) and so the NAD box was ticked. The next day (21st January) basic observations were made of amount of food eaten, temperature and bodyweight, and again the NAD box checked. However, towards the end of the that day, the coat became starey (ruffled), the body temperature rose, and the breathing became more rapid. By the next morning, there was a significant bodyweight loss (12%) which increased during the day to 18% - a strong indication that the animal had not eaten or drunk much, if anything, and that it probably had diarrhoea. In fact there were so many abnormal clinical signs that it was decided to kill the animal on humane grounds before the end of the experiment. The sudden appearance of diarrhoea and the concomitant rapid weight loss and dehydration, laboured breathing, posture, lack of a red light response, etc all confirmed that the animal was becoming severely physiologically compromised and was not going to yield valid results in relation to the scientific objective. Even more significantly, its temperature was now at 35.1°C - a very poor sign, and the extremities were blue (i.e., the colour of the feet and ears). In our experience, this animal would have died that night if not sooner.

This scheme of scoring clinical signs for the recognition and assessment of adverse effects on animals during scientific procedures has been shown to have several advantages and include the following.

- Closer observation of animals is now carried out by all staff at critical times in the experiment as the sheets have indicated those times that are critical for the animal, and when the animals find their circumstances most aversive.
- Subjective assessments of suffering by staff and scientists are avoided, thereby promoting more fruitful dialogue, as evidence-based opinion becomes possible based on the clinical signs. In a sense they empower the technicians and help them illustrate to less experienced persons why an animal is 'not right'.
- Consistency of scoring is increased as the guidance is clear and the scoring options are limited.
- Single signs or combination of signs can be used to indicate overall severity of the procedure, as well as alleviative therapies or scientific procedures at set points in the experiment (e.g., blood sampling).
- The sheets help to determine the effectiveness of any therapy intended to relieve adverse effects.
- The sheets help to determine which experimental models cause least pain and distress, e.g., by comparing alternative animal models helping in refining the scientific procedures.
- The sheets help to train those inexperienced in the assessment of adverse effects.

This has led to better animal care as well as providing useful scientific information such as the recognition of neurological deficits, times of epilepsy or weight loss, as well as unexpected findings such as urinary retention in a model of renal failure. Furthermore, by picking up signs of poor animal well-being early, we can implement humane end points sooner rather than later, which avoids animals being inadvertently lost from an experiment. In the UK,

where severity limits are imposed on each scientific procedure, the sheet can be used to indicate when such limits have been breached, or are about to be breached, or may have to be reviewed, by the precise observation of the presence of clinical signs. The score sheet system provides a visual aid, opens up discussion between interested parties, and helps focus attention on to the animals' condition throughout the procedures. An analysis of the score sheets can reveal patterns of recovery or deterioration and so gives a better picture of the effect of a procedure on the animals from start to finish. The sheet encourages all involved to observe the behaviour of animals, to recognise normal and abnormal behaviours, which will help in determining animals' responses to various procedures, and this will help the development of refining experimental technique by highlighting the type and timing of any adverse effects. The scoring system has proved to be especially useful with new procedures, or when users are not always sure of what effects a procedure will have. In my experience the literature rarely records adverse effects on the animals, or how to avoid them or measure them and I believe scientists have a moral obligation to do so (Morton, 1992).

We now look more closely at ways of improving our peri-operative care and in some experiments we have found that recovery is slower than it could be if different anaesthetics or analgesics, or intraoperative procedures such as maintaining body temperature or giving warm saline were used. We now try to operate earlier in the day so animals have maximum time under close observation and they can be given more support such as fluid therapy or special diets (eg. jelly, fruit, vegetables) which has proven to save animals lives as well as improve the speed of recovery. Finally, obtaining professional advice by seeking the opinions of experienced laboratory animal veterinary surgeons and animal technicians and stockpersons can be very helpful in determining what clinical signs should be looked for.

There is a philosophical debate about whether it is better to cause more suffering to fewer animals or less suffering for many animals to achieve a scientific end. That situation is not common in practice but the UK law takes the view that the level of individual suffering is what matters and so harms should always be minimised. Higher levels of suffering have to be justified on scientific grounds and not on the saving of other animal lives.

Concluding remarks

Refinement may end with the death of the animal but there are other important situations in which animals can suffer, such as their husbandry, social groupings, breeding conditions, handling, restraint, transport, and euthanasia. It is important that these are addressed for reasons of good welfare and good science (see Gunn, 1993; Tuli, 1994; Wadham, 1996). Much research is needed into the Three Rs especially refinement, and grant awarding bodies should be aware of their responsibilities in this regard not only to review carefully the scientific work, they fund, but also to fund research into refinement. Scientists must also write up their work fully and promptly in order to avoid others repeating the work even after a literature search.

Some may argue that the intensive monitoring of animals described above adds to the cost of the research, and of course it does. But scientists have a moral duty to inflict as little suffering as possible and such refinement is the price we should be willing to pay as a compassionate society, and as humane scientists.

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Table 1. Animal Score Sheet (blank) for streptozotocin diabetes model

RAT no.		**ANIMAL ISSUE No.**					
DATE OF STARVING:		**PRE-STARVED WEIGHT:**					
DATE							
DAY							
TIME							
FROM A DISTANCE							
Inactive							
Isolated							
Walking on tiptoe							
Hunched posture							
Starey coat							
Type of breathing *							
Grooming							
ON HANDLING							
Not inquisitive and alert							
Not eating							
Not drinking							
Bodyweight (g)							
% change from start							
Body temperature (C)							
Pale or sunken eyes							
Dehydration							
Diarrhoea 0 to 3 (+m or +b)**							
Distended abdomen / swollen							
Vocalisation on gentle palpation							
Nothing abnormal detected							
Given 5 ml saline s/c ro p.o.							
Other signs noted:							
SIGNATURE							

Special husbandry requirements:
Animals should be kept on grid cage with tray and cleaned twice daily, and rat box for enrichment
Two bottles should be provided for each cage and filled twice daily
Deprivation of water overnight may be sufficient to cause death by dehydration.
Autoclaved diet must be provided.

Scoring details
* Breathing: R = rapid; S = shallow; L = laboured; N= Normal
**0=normal ;1=loose faeces on floor; 2=pools of faeces on floor; 3 = running out on handling;
+ 'm' = faeces contain mucus; + 'b' = faeces contain blood

Humane End Points and actions
1. Any animals showing signs of coma within the first 24-48hr will be killed.
2. Any animals weighing less than the starting weight after 7 days will be killed
3. Any animal showing tiptoe or slow ponderous gait will be killed.

Inform scientist, named veterinary surgeon and animal technician in day to day care if any of 1 to 3 above are seen

Scientific Measures
Animals that have to be killed should have their kidneys placed in formal saline and the pots clearly labelled.

Table 2. Animal Score Sheet (completed) for heterotopic kidney transplant

RAT no. HN1			**ANIMAL ISSUE No.** 234					
DATE OF OPERATION: 20th June at 11.00 hr			**PRE-STARVED WEIGHT:** 250g					
DATE	20	20	21	21	22	22	22	22
DAY								
TIME	13.30	17.30	8.00	4.00	8.00	11.00	14.00	17.20
FROM A DISTANCE								
Inactive	—			—	- / +	+	+	—
Inactive? Try red light response ^						—	+	+
Isolated	—			—	+	+	+	—
Hunched posture	+			+ / -	- / +	+	+	—
Starey coat	—			+	+	+	+	—
Rate of breathing		54			60	64	70	40
* Type of breathing						R	R	L
ON HANDLING								
Not inquisitive and alert		—			—	—	—	+
Eating / Jelly Mash? amount eaten		—		50%	—	?	?	?
Not drinking	—			—	?	?	?	—
Bodyweight (g)	254		260	250	221	215	205	
% change from start		—		6%	0%	-12%	-14%	-18%
Body temperature (C)		35	36.5	37	38	38	36.5	35.5
Crusty red eyes / nose		—			—	—	+	+
Excessive wetness on lower body		—			—	—	—	+
Sunken eyes ^	—			—	—	—	—	—
Dehydration ^	—			—	—	+	+	—
Coat / wet soiled	—			—	—	—	+	—
Pale eyes and ears	—			—	—	—	—	—
Blue extremities ^	—			—	—	—	+	—
Stitches OK? / Date removed:		—			—	—	—	—
*** Swelling of graft ^		—			—	—	—	- / +
NAD		✔	✔	—	—	—	—	—
Dosing								
OTHER DIARRHOEA ^						+	+	
SIGNATURE								

Special Husbandry Requirements
Animals should be put on a cage liner with tissue paper and a small piece of VetBed

Scoring details:
* Type of breathing: R = rapid, S = shallow, L = laboured, N = normal
** Eaten / Jelly mash - amount? - Record as 0 / 25 / 50 / 75 / 100 %
*** Swelling score: 0 = Normal; 4+ = rejection and large swelling

Humane End-Points and actions:
1. Weight loss of 15% or more inform the investigator, veterinarian and intensive care technician.
2. Pre-moribund state (indicating a failing graft).
3. Any major clinical sign recurs after 24 hr (marked ^, less than 35^{0}C).

Scientific measures
Take 1 ml of blood and urine, if possible, - place at 40^{0}C
Place transplanted kidney into 10 ml formal saline.

experiments on animals without inflicting additional pain and suffering.

Institutional Animal Ethics Committees

Each institute permitted to perform animal experiments is usually required to establish an Animal Ethics Committee. In the event that an institution is licensed to perform animal experiments but is not permitted to establish its own Animal Ethics Committee, such as in those institutions in which the number of animal experiment are few, applications for approval to perform animal experiments are reviewed by the National Committee.

Composition

The institutional Animal Ethics Committee shall be comprised of at least 3 individuals, one of which must be a veterinarian. The veterinarian in almost all instances is the same veterinarian employed by that institution. The other members must include a person representing either life or medical sciences and a person whose background is not associated with either life or medical sciences.

Responsibilities

These are:

- to review and approve applications from individuals in which animals are to be used either in experiments or for diagnostic purposes or for teaching;
- to license individuals by conducting courses on the practical aspects of humane animal experimentation;
- to report its activities to the National Committee.

Requirements for Approval to Conduct an Animal Experiment

To obtain approval for an animal experiment, the applicant must:

- provide the relevant background and identify the objectives of the experiment;
- provide full details of the experimental protocol listing the procedures, the animal species, the duration of a single animal experiment and the entire project;
- identify the means that he/she will limit and control the extent of pain and suffering;
- justify the number of animals to obtain meaningful data;
- detail the method of euthanasia;
- provide evidence that an alternate means or technology cannot be used to attain the identical objective.

Conclusions

In contrast to the previous legislation, the revised law addresses the issue of accountability of individuals who perform animal experiments. Furthermore, the Act requires that individuals who intend to conduct animal experiments must be appropriately trained so that experimental animals are treated humanely. Hence, the statute now satisfies many of the concerns of animal welfare organizations.

Arieh Bomzon
Department of Pharmacology
Bruce Rappaport Faculty of Medicine
Haifa, Israel

Videos available from Monash University

In February 1998 Professor Paul Flecknell visited Monash University and other Australian Universities to talk on anaesthesia, analgesia, pain and distress. Monash University has videotaped his talks and these are now available. The cost of each video with notes is $25.

VIDEO 1 (approx. 180 mins)

- Introduction to Anaesthesia (45 mins) - Professor Paul Flecknell, Director, Comparative Biology Centre, University of Newcastle-Upon-Tyne, UK.
- Introduction to Asepsis and Surgical Techniques (45 mins) - Dr Howden, Department of Surgery, Monash Medical Centre.
- Animal Care after Anaesthesia / Surgery (30 mins) - Professor Flecknell.
- Pain and Distress / Analgesia (45 mins) - Professor Flecknell.

VIDEO 2 (approx. 100 mins)

Seminar : How do we really recognise and monitor pain in animals? What does the presence of pain do to our research results? Interactive seminar discussing pain and distress. How pain affects research results, how to monitor and recognise if an animal is in pain / distress, how to intervene and alleviate.

Please order from: Ms Angela Penney, Animal Ethics Officer, Monash University, Clayton Vic 3168. Ph 61-3-9905 5121, fax 61-3-9905 3866 and email: Angela.Penney @adm.monash.edu.au.

Ethics on the move

Professor Peter Singer, who holds a Chair in Monash University's Centre for Bioethics, will take up the position of De Camp Professor of Bioethics at the Princeton University Centre for Human Values in the USA in August, 1999.

Author of the book *Animal Liberation* in 1975, Peter Singer is currently President of Animals Australia, formerly known as ANZFAS.

Book review

From guinea pig to computer mouse. Alternative methods for a humane education.

Edited by U. Zinko,
N. Jukes and C. Gericke.
No ISBN number.
Euroniche, 1997.

This 229-page paperback is a collation of alternative methods to animal use in teaching undergraduate biological science and human and veterinary medicine.

Euroniche is an international network of teachers, students and others working to end the use of animals in undergraduate education. The network extends over more than twenty countries and is coordinated by Nick Jukes (Leicester, UK) and Ursula Zinko (Umea, Sweden).

Most of the book comprises a file of alternatives, listed according to disciplines (e.g., anaesthesia, pharmacology, morphology, surgery). Each discipline is further subdivided and entries are categorised as models, films, video discs, computer programs, simulators or practicums (self-experimentation or other use of humans).

A short description of the alternative is provided, together with details of its cost and availability. Distributors' addresses and sometimes, but not always, telephone, fax and email information are listed in an appendix.

Persons interested in obtaining any of the alternatives listed are asked to contact the Sydney office of the HSI in the first instance, as some of the alternatives are available for evaluation via HSI's alternatives loan program.

One of the difficulties in publishing such a compendium is that the information rapidly becomes dated. As most of the alternatives need to be obtained from Europe or North America, access to them is more difficult.

ANZCCART published a similar monograph in 1993 (*Alternatives to the Use of Animals in Undergraduate Teaching in Australia and New Zealand*), but this is now out of print and will not be reissued. The availability of electronic databases, such as NORINA (see *ANZCCART News* **8**(2):12) as well as other published lists (including that of ANZCCART) is highlighted in the last chapter, as are a number of Internet sites.

The intention of the editors is "to facilitate the introduction of humane teaching aids and to stimulate further interest in alternative teaching approaches".

The absence of an ISBN number or publisher's details is frustrating, as are a number of minor errors in the text. However, it is a useful publication and it is to be hoped it will be updated and expanded, preferably being made available electronically.

Available from Humane Society International Inc. PO Box 439, Avalon NSW 2107. Tel: 61-2-9973 1728; fax: 61-2-9973 1729; email: 100231.2133@compuserve.com or from the United States for US$12.95 (including postage and handling). Tel: 1-301-258 3046; fax: 1-301-258 7760; email: hsuslab@ix.netcom.com.

Robert Baker
Executive Officer
ANZCCART

ANZCCART's third workshop for 1998

This will be held jointly with ANZSLAS (Australian and New Zealand Society for Laboratory Animal Science) and AVASE (Australian Veterinarians Associated with Scientific Establishments) on Monday 21 September in Melbourne, on the day prior to the ANZSLAS Conference.

The theme of the workshop is *Animal health monitoring in the research environment*. It will comprise contributed papers by four speakers, together with a discussion of each paper. The four sessions and speakers are:

- *Animal health monitoring that is cost-effective and produces meaningful results – the balance between too much and too little.* (Dr Bill White, Charles River Laboratories, USA).
- *The monitoring limitations of animals under special circumstances, e.g., micro-isolators, genetically manipulated. Finding real solutions to colony health.* (Dr Patrick Hardy, Charles River Transgenic Services, France).
- *Solving the complexity of importation, quarantine and monitoring of laboratory animals, particularly rodents, for issue in Australia.* (Dr Kevin Doyle, AVA).
- *How do we monitor for the unknown – e.g., emerging organisms found as cell line anomalies? How do we get the feedback we need?* (Dr Earle Steffan, University of Missouri, USA)

The four keynote addresses will form the basis of future ANZCCART facts sheets on effective animal monitoring.

For registration or further information, contact: Ms Carolyn Hand, Monash University Animal Services, Clayton, Vic. 3168. Tel: 03-9905 4872; fax: 03-9905 5736; email: animal.services@adm.monash.edu.au.

1998 ANZSLAS Conference

A very interesting preliminary program for this three-day meeting is now available. To be held in Melbourne from 22 to 24 September, it will feature three overseas speakers:

- Dr Bill White, Charles River Laboratories, USA
- Dr Patrick Hardy, Charles River Transgenic Services, France
- Dr Earle Steffen, University of Missouri, USA

Major topics to be covered include health monitoring, rodent quarantine, models of human disease, primates, occupational health and safety, animal transportation and the design of animal facilities.

For further information, including registration, contact: Ms Carolyn Hand, Monash University Animal Services, Clayton, Vic. 3168. Tel: 03-9905 4872; fax: 03-9905 5736; email: animal.services@adm.monash.edu.au.
URL: http://www.monash.edu.au/aniserv/.

Letters

Embryos

I thought others may be interested in a recent situation that arose at Monash University. A question came up at one of our Animal Ethics Committees (AEC) concerning the use of embryos and ethics approval. If a project involves creating embryos, growing them for a short period *in vitro* and then killing them, does it require ethics approval? Of course AEC approval is required if the eggs and sperm need to be obtained from a live animal, but what if the project involved eggs from the abattoir and frozen sperm left over from an already approved project?

The *Code of Practice* states that, "When fertilised eggs are used, the method of disposal must ensure the death of the embryo" (3.3.24)., thus implying AEC approval is required in all situations. The general consensus from the AEC was that these projects should have approval to ensure the fate of the embryos and that they are killed humanely, since embryos can theoretically become foetuses and then sentient animals.

I was asked by the AEC if other Australian or New Zealand universities have a policy on embryo experimentation and how they deal with this situation, so I posed the question to the ANZCCART email discussion group.

All of the responses I received confirmed that Australian and New Zealand institutions do require AEC approval for embryo work with the following additional justifications:

- the third paragraph of the Scope of the Code, 6th edition, clarifies the matter. "The Code covers all live non-human vertebrates. Eggs, foetuses and embryos must be treated in a humane manner where development of an integrated nervous system is evident. Investigators should consider forwarding proposals to use higher invertebrates to AECs".

- "We regard the production of embryos and their retrieval (which generally involves euthanasia or surgery) as a manipulation, as defined by New Zealand legislation. Therefore, such a procedure requires prior AEC approval".

Comments regarding how to deal with the situation, and if we count these embryos, included:

- "if your group is dealing with embryos at very early cell division stage, well before neural development, I think that counting them is superfluous. The purpose of counting experimental animals for the statistical returns is to note the numbers in different categories which might have suffered as a result of what was done to them. Pre-sentient embryos are not an issue here. It is irrelevant what they might be able to experience once their development has taken its course. The issue is can the animals, foetuses, embryos suffer? If so, they should be counted. If not they should not be counted. The same basis is used for inclusion or exclusion of different species from those which, as conscious animals, must be included when experimented upon. There will obviously be a grey area where more developed embryos, if separate from their dams, should be included in State returns if it is thought probable they might suffer, or should be included if specific measures are taken to ensure that they do not".

Thank you to those who responded. As usual the discussion list was very helpful in providing information to a question.

Angela Penney
Monash University
Clayton Vic 3168

Jugular venous sampling in the rat

This note is a response to the letter published in *ANZCCART News* **10**(1), March 1997 by Graham Mayrhofer, which described a method for obtaining blood samples from rats using the lateral tail vein. The Gore Hill Research Laboratories of the Royal North Hospital and the University of Technology, Sydney, do not allow researchers to obtain blood from any animal from the retro-orbital plexus or by amputative venesection of the tail.

The method described here utilises the jugular vein of the rat and is a reliable method for obtaining one ml of non-haemolysed rat blood, and ensuring survival of the animal. The neck area in the rat is also highly resistant to infection. The easiest rats to sample with this method are young adult male rats, as breast tissue and excess fatty tissue are avoided. In skilled hands this procedure comfortably takes ten to fifteen minutes per rat.

Materials required are a 25 G or 26 G needle, a 1 ml or 2.5 ml syringe, a fine pair of forceps and scissors, suture material (such as 4.0 nylon or vicryl), tape, one swab, a shaver, and access to an inhalational anaesthetic.

The method is to apply inhalational anaesthetic via an individual head box, secure the rat in a supine (lying on back) position with forelimbs outstretched and secured with tape, and shave the area above the clavicle on the rat's right side. Find the uppermost part of the sternum in the midline, then locate a point a half cm above and one cm lateral to this. Make a one cm transverse cut through the skin at this site – the jugular vein pulsation can often be seen at this point. Carefully dissect away the connective tissue until the jugular vein can be clearly visualised and aspirate one ml of blood. Apply pressure for ten seconds to the aspirated area and empty the syringe, without using the needle tip to avoid haemolysis, and apply a single suture to the skin incision, allowing the animal to wake in a recovery box.

Steve Trigg
Kolling Institute
Royal North Shore Hospital
Sydney

ANZCCART News reader survey results

The interim report from the Department of Management at Monash University, which undertook this survey, has now been received and copies provided to the Board. The survey was conducted by Dr Milé Terziovski and Ms Sally Townsend, to whom the Board expresses its thanks.

The survey was sent to 440 readers, of which 100 responded – a response rate of 23 per cent. The general results can be summarised as:

- *ANZCCART News* readership is fairly diverse and covers a range of research, teaching and lay interests. Respondents generally agreed that the newsletter was a reliable periodical, and that editorial and administrative staff were responsive to the needs of their readers.
- Recent layout changes were greeted with enthusiasm. Suggested improvements included the continued use of colour and visual input such as diagrams, charts and photographs, the inclusion of more Facts Sheets, and the introduction of more reports or articles to meet the interests of particular areas of animal care and research.
- There were mixed reactions to the idea of an annual fee to defray production costs. Respondents were more agreeable to suitable advertising being introduced as long as it did not detract from the professional tone of the newsletter.
- There was only a small response to requests for colleague referrals, and other ways need to be considered to increase circulation.

The recommendations were that:

- Changes to the layout and style be maintained and further improved.
- Facts Sheets (e.g., animal welfare, caging issues, native animals) be continued as a regular feature.
- Invitations be extended to readers who have offered to submit articles and reviews for publications.
- The content of the newsletter should include a mixture of long and short articles and information on technical issues.
- Paper mail be maintained and the viability of a combination paper mail and World Wide Web also be investigated.
- Further investigation is required on the introduction of an annual fee.
- Paid advertisements be trialed (12 months) and be confined to professional development, relevant consulting services and reader initiated classified advertisements.
- Increased circulation might be achieved through attracting new readers at employment induction and through TAFE colleges.

The Board is currently considering these recommendations.

ANZCCART Workshop on the Sources of Animals for Research and Teaching

The second workshop for 1998 was held at the Waite Campus of the University of Adelaide on 28 May and attended by 38 persons. Each workshop is intended to explore issues of current interest, drawing on speakers and participants working in the relevant areas.

This workshop examined three areas related to the supply of animals for research and teaching. The first session covered the supply and demand of rats, mice, guinea pigs and rabbits in Australia and New Zealand now and in the future. This session was led by Dr David Pass, Director of the Animal Resources Centre in Perth and current President of ANZSLAS.

The second session was chaired by Dr Robert Baker, Executive Officer of ANZCCART. It discussed other animals used for research and teaching, including wildlife, zoo animals, production animals, pound animals and primates. It included discussion of access to and subsequent release of native species, the use of zoo animals as an educational resource, the problem of access to pound animals for training veterinary students in anatomy and surgery and the particular problems associated with housing primates, which has been addressed by the NHMRC.

The last session, chaired by Mrs Lynne Milne, of Landcare, Christchurch, examined the particular issue of research relating to vertebrate pests, from both an Australian and New Zealand perspective. Species discussed included brushtail possums, rabbits and hares.

Twelve speakers contributed to a very interesting workshop. It was agreed that a working party of Dr Pass, Professor Mike Rickard (ANZCCART Chairman) and Dr Robert Baker would convene before the September ANZSLAS Conference, to discuss mechanisms to better coordinate the production and supply of laboratory animals in Australia.

Notes from the meeting will be provided to all participants and other interested persons. Copies of these, as well as the notes from the previous workshop on the *in vitro* production of monoclonal antibodies, are available free of charge from ANZCCART's Adelaide Office.

EU Report on pig welfare

This 190 page report of the European Commission's Scientific Veterinary Committee entitled *The Welfare of Intensively Kept Pigs* is available in hard copy or on disc, to which has been added extra information on pig welfare. The cost is UK£90.00 or US$150.00, including postage. Issues considered include possible future pig welfare legislation in the EU; what aspects of pig welfare are currently of concern, and will segregated early weaning and genetic enginering be restricted? Contact: Pig Disease Information Centre, Bar Road, Lolworth, Cambs, CB38DS, UK. Fax: 44-1954 780 235; email: pdic@btinternet.com.

ANZCCART to hold two conferences in 1999

ANZCCART will hold conferences in both Australia and New Zealand next year.

The Australian conference, whose theme is to be *The Use of Wildlife for Research*, will be held at the Western Plains Zoo, Dubbo, NSW on Wednesday and Thursday, 26 and 27 May, 1999. The Zoo has an excellent conference facility seating 200 people and a very interesting program is anticipated. Dubbo is serviced regularly by air from Sydney.

The Western Plains Zoo opened in 1977 and is administered by the Zoological Parks Board of NSW. Its Director, Dr Ian Denney, is assisting with the program as well as with local arrangements.

The New Zealand Board of ANZCCART is working in partnership with the New Zealand Animal Welfare Advisory Committee (AWAC) to jointly mount a major conference in New Zealand in late November 1999. The venue for the conference is Te Papa Tongarewa, the new Museum of New Zealand, situated in Wellington.

The joint programme planning committee consists of Dr Mark Fisher (ANZCCART New Zealand / AWAC planning committee chair), Dr David Bayvel (AWAC / MAF), Professor Neville Gregory (AWAC), Dr Ken McNatty (ANZCCART New Zealand), Professor David Mellor (ANZCCART New Zealand) and Mrs Gill Sutherland (ANZCCART New Zealand).

The proposed conference title is *Innovation, Ethics and Animal Welfare: Public Confidence in Science and* Agriculture. The first day will deal with *Farming Animals in 2020: Issues and Options*, and will have two main purposes:

- to consider how application of knowledge has affected the welfare and productivity of farmed animals during the past century, and how it might affect different livestock sectors in the future; and
- to consider market trends and consumer preferences which will affect farming during the next twenty years.

The second day, provisionally entitled *Science and Trust: Innovation on the Edge*, will have several major purposes:

- to explain in lay terms cloning, gene technologies and between-species transplantation of tissues or organs;
- to anticipate the future applications of such technologies and consider their benefits and pitfalls;
- to consider how innovation creates ethical dilemmas, excitement and fear; and
- to explore the impacts the media and regulation can and do have on public trust in science.

Coming up

Biology of Animal Stress Conference
16 - 19 August, 1998
University of California, Davis
Contact: www.aes.ucdavis.edu/AnStress.html
Email: AnStress@agdean.ucdavis.edu

LAWTE ' 98 Meeting
20 - 21 August, 1998
St Louis, Misouri
Contact: PRIM&R
Tel: 1 617 423 4112
Fax: 1 617 423 1185
Email: PRMR@aol.com

Endocrine Society of Australia / Australian Society for Reproductive Biology Conference
24 - 26 August, 1998, Perth
Contact: Dr Tom Ratajczak
Tel: 08 9346 2596
Fax: 08 9346 3221

Invitox 98
10th International Workshop on In Vitro Toxicology
14 - 18 September, 1998
Winchester, UK
Contact: Dr Diane Benford
School of Biological Sciences, University of Surrey, Guildford, Surrey GU2 5XH, UK
Fax: 44 1 483 300 374
Email: d.benford@surrey.ac.uk

Australian and New Zealand Society for Laboratory Animal Science 22nd Annual Conference
21 - 24 September, 1998
Melbourne
Contact: Dr Susan Maastricht
Tel: 03 9905 4871
Fax: 03 9905 5736
Email: susan.maastricht@adm.monash.edu.au

AALAS Annual Meeting
25 - 29 October, 1998
Cincinatti, Ohio
Contact: AALAS
Tel: 1 901 754 8620
Fax: 1 901 754 2546
URL: http//www.aalas.org

2nd International Conference on Transgenic Animals
26 - 29 October, 1998
Beijing, China
Contact: BILONG Transgenics
Fax: 86 10 6253 2114
Email: bilong@public.east.cn.net or libr@mimi.cnc.ac.cn or bilong@post.tau.ac.il
URL: http://www.freeyellow.com/members/bilong/

19th SETAC Annual Meeting
15 - 19 November, 1998
Charlotte, North Carolina
Contact: SETAC office
Tel: 1 904 469 1500
Fax: 1 904 469 9778
Email: setac@setac.org

Australian Society for Medical Research Conference
22 - 25 November, 1998
Hobart
Contact: Conference Design
Tel: 03 6224 3773
Fax: 03 6224 3774
Email: conf.design.hba@trump.net.au

International Conference on the Use of Humane Endpoints in Animal Experiments for Biomedical Research
23 - 25 November, 1998
Zeist, The Netherlands
Contact: Bjorn Steen
RIVM, Central Animal Laboratories
Tel: 31 30 274 2503 / 2377
Fax: 31 30 274 4408
Email: Bjorn.Steen@RIVM.nl

Australian Society for Immunology Conference
29 November - 3 December, 1998
Melbourne
Contact: Convention Associates
Tel: 03 9887 8003
Fax: 03 9887 8773
Email: ca@netwide.com.au

News

News from the NHMRC Animal Welfare Committee

Dr Alana Mitchell has recently accepted the position of Animal Welfare Liaison Officer with the National Health and Medical Research Council. This is a new position, created by the NHMRC to meet the need for more effective liaison with animal welfare groups, lobby groups and the wider community, particularly where concerns exist about animals used in NHMRC funded experiments. One of her duties is to keep a record/database of perceived problems with the Code of Practice, to be collated and summarized for use by the committee which next reviews the Code of Practice.

Until December 1997, Alana was a Senior Research Officer at the Baker Medical Research Institute. She was a molecular biologist, working on genes for proteins with a role in cholesterol transport around the body. A member of the Alfred Hospital and BMRI Animal Experimentation Ethics Committee for eight years and chair of the committee in 1996 and 1997, Alana is pleased to have the opportunity to continue and extend her commitment to animal welfare in her new role.

Alana sees herself as a mediator or negotiator in situations where there may be conflicting views. She is quick to point out that her brief from Dr Mike Calford, Chair of the Animal Welfare Committee, is to use her discretion about whether to report back to the NHMRC Animal Welfare Committee on any issue. She hopes to be regarded as a sort of "ombudsman", in that she is keen to hear from anybody with a grievance about any aspect of animal welfare, so that issues can be openly discussed and resolved. In her view, "the worst thing would be for dialogue between concerned parties to break down". Keen to establish a close working relationship between NHMRC and ANZCCART, Alana sees such interaction as beneficial to animal welfarists, researchers and all groups with an interest in the well-being of animals.

Alana can be contacted at the following: Phone: (03) 9380 4483; fax: (03) 9381 1703; email:alanam@netspace.net.au Postal address: c/- Science Link Pty Ltd, PO Box 46, Parkville Vic. 3052.

New ethical review process for UK animal-based research

The UK Home Office, which administers the 1986 *Animals (Scientific Procedures) Act*, has decided that every establishment wishing to carry out animal research will need to set up an Ethical Review Process by 1 April, 1999.

This follows a report from an October, 1997 workshop, *Progressing the Ethical Review Process*, initiated and co-convened by the Research Animals Department of the RSPCA (UK). The aim of the workshop, held at the Wellcome Trust in London, was to develop the concept of local ethical review processes as set out by the Home Office in its letter to Certificate Holders in April, 1996. This letter canvassed various methods of local review processes such as:

- ethics committees;
- care and use committees;
- the named veterinary surgeon;
- project refinement reviews;
- other awareness raising activities, such as staff training and provision of seminars on ethics and welfare.

Hugo van Poelgeest Prize awarded

On February 16, the Hugo van Poelgeest award was presented to Dr Geny Groothuis. The prize of approximately US $10,000 is awarded every four years to an investigator from the Netherlands who succeeds in developing methods leading to outstanding scientific results without the use of animals, in fields where animal experimentation is in use.

Dr Groothuis is a senior scientist at the Department of Pharmacokinetics and Drug Delivery, Groningen Institute of Drug Studies, Groningen University. She was awarded the prize because of her outstanding research for the development of *in vitro* test systems with human tissue to predict organ-specific drug metabolism and toxicity in man in an early phase of drug development. Techniques are being devloped for preservation of scarcely available human tissue and for the preservation and incubation of slices of lung, liver, kidney and intestinal tissue.

The development of an integral *in vitro* test system results in an infrastructure that contributes to a more adequate choice of species for metabolism and toxicity studies and a reduction in the use of experimental animals.

The research is subsidised by the Netherlands Alternatives to Animal Experiments Platform. For further information, contact: Dr GMM Groothuis, Dept of Pharmacokinetics and Drug Delivery, Groningen Institute of Drug Studies, Ant. Deusinglaan 1, 9713 AV Groningen, tel: +31 50 363 6414, fax: +31 50 363 3247, email: G.M.M. Groothuis @farm.rug.nl.

ANZCCART News is published quarterly by the Australian and New Zealand Council for the Care of Animals in Research and Teaching Limited.

It is a publication for researchers and teachers; members of animal ethics committees; staff of organisations concerned with research, teaching and funding; and parliamentarians and members of the public with interests in the conduct of animal-based research and teaching and the welfare of animals so used.

Contributions to ANZCCART News are welcomed and should be sent to:

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http://www.adelaide.edu.au/ANZCCART/

or

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ISSN 1039–9089