Humane endpoints in animal experimentation for biomedical research: ethical, legal and practical aspects

David B. Morton
Centre for Biomedical Ethics, Division of Primary Care, Public and Occupational Health, School of Medicine, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK
[e-mail: d.b.morton@bham.ac.uk]

Summary

The reasons for implementing humane endpoints are discussed and include: when the scientific results will no longer be valid; when there is a lack of proportionality between suffering and benefit; when the suffering has exceeded a humane limit regardless of benefit; and finally, when surrogate endpoints could be employed. A scheme for the recognition and assessment of clinical signs and their use in determining humane endpoints is put forward, and some examples are given.

The ethical, legal and practical aspects of refining animal experimentation will be a recurrent theme during this meeting, particularly with reference to the development and implementation of humane endpoints. I would also add to that list, scientific aspects, for the reason that sometimes a humane endpoint will revolve around the scientific validity of an individual animal undergoing a specific scientific procedure. I will address some very general issues over the four aspects I have just mentioned, but in particular I will cover the use of score sheets for the recognition and assessment of the effects on an animal of an experiment, and the use of score sheets in the implementation of severity limits and endpoints.

Ethical aspects

Some of the ethical arguments that underpin animal experimentation are whether we should use animals at all, and if so, for what purpose. The use of animals for testing cosmetic products and ingredients has now been prohibited in the UK, but it is generally accepted that animal use for medical benefit is, by and large, justifiable. However, in all cases, animal suffering should be reduced to the minimum consistent with attaining the scientific objective and the ethical framework of the Three Rs devised by Russell and Burch (1959) is the most widely quoted and applied. Out of replacement, reduction and refinement, it is the latter which will concern us most during this conference. Refinement has been defined as: 'Those methods which avoid, alleviate or minimize the potential pain, distress or other adverse effects suffered by the animals involved, or which enhance animal well-being' (Morton 1998a).

Categories of humane endpoints and scientific aspects

As much of the process of animal experimentation causes animals to suffer (even their husbandry is often of some concern) and as all such suffering could be described as inhumane, more humane endpoints should, perhaps, be seen as 'less-inhumane endpoints' (see Balls 1999). Another way to look at them is in the light of 'avoidable' suffering and to go beyond what is required to achieve a scientific objective could be described as inhumane and unnecessary. One can identify

five types of humane endpoints, the first two relate to scientific aspects.

First, when an animal is no longer going to provide scientifically useful information because it is so physiologically deranged which is sometimes related, but not always, to the variable being studied (e.g. an animal may develop acute unremitting diarrhoea during the course of a test and so become metabolically unstable; another example may be an intercurrent infection).

A second type is when an animal is no longer going to provide scientifically useful information because it is so psychologically disturbed—again sometimes related, but not always, to the variable being studied (e.g. an animal becomes difficult to dose and is distressed to the point where it affects its mental and hence its metabolic state, and we know that the central nervous system (CNS) directly affects immune function; the system of husbandry can affect CNS neuroreceptors: see Wadham 1996).

A third type of humane endpoint is when the suffering caused to animals during a study is higher than predicted and so on a cost–benefit analysis there is a loss of proportionality in that the harms done to animals are not outweighed by the benefits being sought.

Fourth, when the level of suffering is so high that it is simply wrong to cause that degree of harm to an animal—in UK law and OECD guidelines this would be termed as 'severe pain and severe distress'.

Fifth, when a high degree of suffering can be justified but there is no need to cause that level as earlier pre-lethal even pre-painful endpoints can predict a scientific endpoint—I have called these surrogate endpoints (see Cussler *et al*. 1999, Hendriksen *et al*. 1999); other examples may be using blood glucose levels rather than death for studies on transplant therapies for diabetes, or terminating a test to categorize a substance as soon as the first positive is obtained if that would ultimately determine its label even if the remaining animals were negative. It is in this area that experimental design can be critical (Morton 1998c, Fry 1999).

Whilst a reduction in the number of animals used in research is a consideration, most cultures place little emphasis on the value of an animal's life and so there are few controls on the numbers killed other than the actual method of killing (see Hansen *et al*. 1999). One might stop for a moment to contrast the present debate over what to do with the 1800 or so chimpanzees in captivity, with an absence of debate surrounding the killing of other non-human primates. I am not so convinced that taking a mammal's life is not a moral harm (as others have also argued e.g. DeGrazia 1996), but in any event I would rank it third of the Three Rs. It must also not be forgotten that the ways in which we house animals may also cause them to suffer and that discomfort and mental distress in husbandry is another very important area for refinement.

Legal aspects

As far as I am aware, all national and international laws as well as international guidelines incorporate the Three Rs concept. Clauses in such laws deal with: always using non-sentient replacement methods if readily available; carrying out research under anaesthesia throughout whenever possible; using the least sentient species on the phylogenetic scale; giving adequate analgesia e.g. after surgery; obtaining professional advice from veterinarians and other competent persons whenever needed; ensuring training and competence of all personnel; the purpose breeding of some species and for keeping all stock and experimental animals in adequate environmental conditions; minimizing pain and suffering and reducing the number of animals to the fewest necessary; giving adequate justification for the research in the first place; and if animal suffering is going to be caused which is possible to relieve but the scientific objective prohibits such alleviation, then that suffering will require rigorous justification.

Practical aspects

The practical aspects of refining animal experiments relate to when they may be experiencing pain, dystress (stress under which an animal fails to thrive or cope—see

Morton 1995a, 1998a), mental distress, discomfort or lasting harm. Before any of these states can be alleviated or estimated, or experiments refined in any way to cause less pain and suffering, we have first to be able to recognize when animal well-being is being affected, both positively as well as negatively. Moreover, it is important to eliminate any animal suffering in order to achieve science of high quality and specificity in relation to the scientific question being asked, as well as to practise humane science at the least economic cost (see Claassen 1994, Balls *et al*. 1995, Morton 1998b).

One way to approach this problem is to use a list of key clinical signs (score sheets—see Morton 1990, Morton & Townsend 1995, Morton 1995b, 1997a,b) as a way of determining the degree to which an animal's physiology and mental state has deviated from normal, and then to use this perturbation for the assessment of severity. Score sheets have to be drawn up specifically for each scientific procedure and for each species and sometimes even for each strain undergoing that procedure; i.e. they can rarely be generalized. The sheets list the cardinal clinical signs that are observable and measurable and are developed through the experience of a team of observers (see Morton *et al*. 1990). A team approach should be adopted and the animal caretakers will be crucial in this regard as they are the most likely to know when an animal is 'not right', as indicated by, e.g. a change in behaviour, posture, appearance or even the feel or smell of an animal. The veterinarian's input should help in identifying objective clinical signs as well as the biology of the species to target a range of relevant behavioural and physiological responses, and the scientists should be conversant with the perturbations that might be expected during an experiment due to the scientific paradigm. All these factors will be important guides in the assessment of the effects of a scientific procedure on an animal. By detailing the cardinal signs of any particular protocol and regularly observing animals at critical periods during the experiment, an objective assessment of animal well-being can be made throughout the experimental period.

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 key signs that most animals will show during that experiment, and that are relevant to the assessment of suffering, is made. These cardinal signs are set out against time in the score sheet (an example is given in Table 1). Crucially, any clinical sign has to be reduced to a level which reduces the scope for observer interpretation and 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, i.e. within the normal range, and positive signs indicating that the animal is outside the normal range. In this way it is possible to scan a score sheet to gain an overall impression of animal well-being: the more plusses, the more an animal has deviated from normal with the inference that it is suffering more than before. Practically, it is important to develop a disciplined 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 its appearance noted. Next, as the observer approaches the pen or removes the cage lid, the animal will inevitably start to interact with the observer and its response can also be assessed for normality (it may have become more aggressive or fearful, or even vocalize). Finally, a detailed clinical examination can be carried out by handling and restraining the animal in some way and observing its appearance carefully as well as making any relevant clinical measurements e.g. body weight, temperature, in addition to its behaviour.

At the bottom of the sheet there are guidance notes for animal caretakers and veterinary technicians about what they should provide in terms of husbandry and care for animals undergoing that scientific procedure. There are also guidelines on how to record qualitative clinical signs (such as diarrhoea and respiration) as well as criteria by which to implement humane endpoints. Finally, if an animal has to be killed there are instruc-

Table 1 Score sheet for heterotopic heart transplant. Licence No: 1234. Rat

Rat No: IR2	Issue No: 5390					
Date of operation: 27/1/96	>Preoperation weight: 300					
Date	27 Jan	28 Jan	28 Jan	28 Jan	29 Jan	29 Jan
Day	0	1	1	1	2	2
Time	16:30	8:30	12:00	16:30	8:30	11:00
From a distance						
Not active	−	−	−	−	−	+
If inactive—red-light response Y/N	−	−	−	−	−	N
Isolated	CS	Gp'd	−	−	+	+
Huddled	−	−	−	−	−	−
Hunched posture	+/−	−	−	−	+	+
Pinched abdomen	−	−	−	−	−	−
Starey coat	+/−	−	−	+	+	+
Rate/*Type of breathing		60N	60N	70R	85R	85L
Not grooming	−	−	−	+	+	+
Tiptoe walking	−	−	−	−	−	−
On handling						
Not inquisitive or alert	−	−	−	−	−	+
No righting reflex	−	−	−	−	−	+
Not eating/drinking	−	−	−	−	+	+
Bodyweight (g)	300	295	290	291	265	250
% change from start			3.30%		11.60%	16.70%
Body temperature (°C)	37.3	37.8	37.4	38.1	36.1	35.1
Crusty red eyes/nose E/N	−	+N	+N	+N	+E.N	+E.N
Orifices soiled (diarrhoea or urine)	−	−	−	−	+D	+D
No faeces	−	−	−	−	−	−
Diarrhoea	−	−	−	−	+	+
Dehydration—square tail/skin pinch	−	−	−	−	+	+
Backbone nobbly	−	−	−	−	−	−
Blue extremities	−	−	−	−	+	+
Vocalization	−	−	−	−	−	−
Dosed (Y/N) MTX 1 mg/kg	N	Y	N	N	Y	N
Other						Killed
***Donor heartbeat	4+	4+	4+	4+	4+	4+
NAD	√	√				
Signature:	INR	KS	INR	INR	KS	INR

Special husbandry requirements:
Recovery room, vetbed required, may require heat
Scoring details:
*Breathing: R = rapid; S = shallow; L = laboured; N = normal
*** Donor heartbeat: 4+, 3+, 2+, 1+, R (4+ = very good)
Humane endpoints and actions:
1. Inappetance, diarrhoea, dehydration for more than 48 h—animal to be killed
2. Weight loss of 20% or more inform researcher, animal welfare officier and vet; more than 25% to be killed
Scientific measures:
On culling remove heart graft, spleen, thymus, small bowel, bone marrow, take 1 ml blood

tions about what other actions should be taken, such as tissues to be retrieved or placed in formal saline; this helps ensure that the maximum information is always obtained from any animal in a study. While these sheets take time to fill in, it is not difficult for an experienced person to see if an animal is unwell, so the 'nothing abnormal detected' (NAD) box is simply checked. However, if an animal is not normal, it does take time to check it and to make judgments over what actions to be taken, but is that not the price for practising humane science?

Interpreting the score sheet

It can be seen from the completed example score sheet that there are more pluses to the

right than to the left but 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 (28 January) basic observations were made of the amount of food eaten, temperature and body weight, and again the NAD box checked. However, towards the end of 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 body weight loss (12%) which increased during the day to 17%—a strong indication that the animal had not eaten or drunk much, if anything, and that it probably had diarrhoea. In fact, by then 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, abnormal 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°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 despite any resuscitative measures.

Technician-in-charge

In order to promote good care and good continuity of care, we allocate an animal technician/caretaker or veterinary technician to be responsible for liaising with scientists and other technical staff, and to maintain and update the score sheets. The roles of the technician-in-charge are: to check that the appropriate licenses and approved protocols are in order and to cross-check them with what the scientist actually intends to do that day to the animal(s); to check the score sheet is appropriate before an experiment begins; to know the purpose of the experiment and the scientific objectives; 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 (caretakers, technicians, scientists, veterinarians) know how to use the score sheets and can recognize the clinical signs and can interpret the signs clearly into humane endpoints; to check that caretakers and technicians not familiar with that experiment, say doing a weekend or holiday rota, are informed about animals; to liaise with scientists over the experiment e.g. timing, numbers of animals, equipment, endpoints; to update the score sheets based on new signs or combinations of signs observed; and to report to the responsible persons (e.g. the veterinarian, senior scientist) any concerns over the animals or personnel involved.

We have found that the advantages of score sheets include: a closer observation of animals by all staff at critical times as the sheets have indicated the times when animals find their circumstances most aversive; subjective assessments of suffering by staff and scientists are avoided, promoting more fruitful dialogue as evidence-based opinion becomes possible based on the clinical signs (in a sense they empower the technicians by helping 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; the effectiveness of any therapy intended to relieve adverse effects is better evaluated; helping us to choose from two or more alternative experimental models the one that causes least pain and distress etc. thus helping to refine scientific procedures; helping us to train those inexperienced in the assessment of adverse effects or in that particular scientific procedure; and have enabled us to carry out a retrospective analysis of the adverse effects of any scientific procedure and its degree of severity. Moreover, score sheets have indicated single signs or a combination of signs that can be used to indicate overall severity of the procedure, as well as alleviative therapies or scientific procedures at set points in an experiment (e.g. blood sampling) and to determine humane end-

points. The sheets have also been found to add to the science as more careful observation of animals is carried out. An analysis of score sheets has revealed patterns of recovery or deterioration and, as a consequence, has given a better picture of the effect of a procedure on the animals from start to finish.

The sheets are constantly being developed and updated with further experience and it is surprising how the process never seems to stop as new staff pick up new signs, or new signs develop as the experimental model is slightly modified. Staff also start to perceive patterns of adverse effects that, when taken as a whole, indicate early death or early deterioration sufficient to warrant the animal being killed on scientific grounds alone. Such information has led to better animal care as well as providing useful scientific information such as the recognition of neurological deficits, times of epilepsy (in one study we found that fits occurred mainly at night), or weight loss, as well as unexpected findings such as urinary retention in a model of renal failure.

Severity limits and their implementation

In the UK, under the Animals (Scientific Procedures) Act 1986, each scientific procedure has a severity limit and the question is how that limit is recognized in practical terms. In theory there are four recognized severity bands: mild, moderate, substantial and severe, but neither severe pain nor severe distress is permitted under any circumstances. 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 (see Morton & Griffiths 1985, Morton & Townsend 1995, Olfert 1995, 1996, Morton 1995b, 1997a,b, 1998a,b, CCAC 1998). Severity limits can be interpreted as the degree of deviation from normality along with other indicators of health and quality of life. Take body weight as an example. A body weight loss of up to 10, 20 or 25%, or greater than 25% in a few days compared with controls can be used to interpret mild, moderate, substantial and severe respectively. However, body weight alone may not be adequate as animals with tumours or ascites may increase in body weight but lose body condition (i.e. muscle mass) and be experiencing extreme suffering. Ullman-Culleré and Foltz (1999) have recently developed a good way of estimating body condition in mice. 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, therefore, that a holistic approach is taken so that clear clinical signs can be used to determine humane endpoints in accordance with the scientific benefit and humane research.

Humane endpoints

In the past it was not uncommon to use death as an endpoint (van den Heuvel *et al.* 1990). However, death is rarely related to the experimental variable under study, but rather to indirect effects such as dehydration and starvation by animals not being able to drink or eat. Dehydration leads to haemoconcentration and an increased viscosity of the blood which the heart cannot cope with and so leads to heart failure. Inadequate food intake in rodents can lead to low body temperatures and death. An animal may take several days to die and so surrogate lethal endpoints need to be established (Mellor & Morton 1997). One approach is to note the clinical signs preceding death closely and to determine those signs which are shown to be irrevocably linked with death and to use such signs as pre-lethal endpoints. 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 endpoint can be relied upon. Grant awarding bodies have a moral obligation to support research developing such humane endpoints. This approach can be used in: toxicity testing; the testing of medicines such as vaccine potency testing; the rodent protection test for novel antibiotics; virulence assessments for micro-organisms or parasites; batch testing of

natural or synthetic products, etc. Cussler *et al*. (1998) 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 found that animals showing slow circular movements invariable progressed to death—the traditional endpoint for the test—and could be reliably used instead. Soothill *et al*. (1992), in an investigation into the effectiveness of phages for resistant Staphylococci, showed that animal suffering could be reduced by several hours by taking a body temperature of less that 35°C as a pre-lethal endpoint (see also Cussler *et al*. 1999 and Hendriksen *et al*. 1999 for other examples of surrogate endpoints).

Conclusion

The score sheet 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 or measure them and scientists have a moral obligation to do so (Morton 1992). We are now looking more closely at ways of improving our animal care and this has proven to improve the science, and we do not lose animals inadvertently. The sheets encourage all involved to observe the behaviour of animals, and to recognize normal and abnormal behaviours, which will help in determining animals' responses to various procedures. This in turn will help us to devise ways of refining experimental technique by highlighting the type and timing of any adverse effects, and to develop surrogate endpoints for death or substantial severity. No money is needed to implement many of the ideas in this paper however, the right attitude of the whole of the research team is essential if those involved wish to claim their use of animals does not cause unnecessary suffering.

References

Balls M, Goldberg AM, Fentam JH, *et al*. (1995) The Three Rs: the way forward. The Report and Recommendations of ECVAM Workshop 11. *Alternatives to Laboratory Animals* **23**, 838–66

Balls M (1999) The biomedical sciences and the need for less inhumane animal procedures. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 1–4

CCAC (1998) *Guidelines on: Choosing an Appropriate Endpoint in Experiments Using Animals for Research and Testing*. Available from Canadian Council on Animal Care, Constitution Square, Tower II, 315–350 Albert Street, Ottawa, Ontario, Canada K1R 1B1

Claassen V (1994) Neglected factors in pharmacology and neuroscience research. biopharmaceutics, animal characteristics, maintenance, testing conditions. In: *Techniques in the Behavioural and Neural Sciences*, Vol. 12. (Huston JP, ed). Amsterdam: Elsevier Science

Cussler K, Morton DB, Hendriksen CFM (1998) *Klinische Endpunkte als Ersatz fur die Berstimmung der Letalitatsrate bei Tollwutinfektionsversuchen zur Impfstoffprufung. Altex* (*Alternativen zu Tierexperimenten Supplement*) **98**, 40–42

Cussler K, Morton DB, Hendriksen CFM (1999) Humane endpoints in vaccine research and quality control. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 95–101

DeGrazia D (1996) *Taking Animals Seriously: Mental Life and Moral Status*. Cambridge: Cambridge University Press

Fry DJ (1999) Relating criteria for humane endpoints to objectives. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 54–7

Hansen AK, Sandøe P, Svendsen O, Forsman B, Thomsen P (1999) The need to refine the notion of reduction. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 139–44

Hendriksen CFM, Steen B, Visser J, Cussler K, Morton D, Streijger F (1999) The evaluation of humane endpoints in pertussis vaccine potency testing. In: *Humane Endpoints in Animal Experiments for Biomedical Research* (Hendriksen CFM, Morton DB, eds). London: Royal Society of Medicine Press, pp 106–13

Mellor DJ, Morton DB (1997) Humane endpoints in research and testing. Synopsis of the workshop. In: *Animal Alternatives, Welfare and Ethics* (van Zutphen LFM, Balls M, eds). Amsterdam: Elsevier Science, pp 297–9

Morton DB, Griffiths PHM (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. *Veterinary Record* **116**, 431–6

Morton DB (1990) Adverse effects in animals and their relevance to refining scientific procedures. *Alternatives to Laboratory Animals* **18**, 29–39

Morton DB. Burghardt G, Smith JA (1990) Critical anthropomorphism, animal suffering and the ecological context. *Hasting's Center Report Spring Issue on Animals, Science and Ethics* **20**, 13–19

Morton DB, Townsend P (1995) Dealing with adverse effects and suffering during animal research. In: *Laboratory Animals–An Introduction for Experimenters*, revised edn (Tuffery AA, ed). Chichester: John Wiley & Sons Ltd, pp 215–31

Morton DB (1992) A fair press for animals. *New Scientist* 11 April 1992, No. 1816, pp 28–30

Morton DB (1995a) Recognition and assessment of adverse effects in animals. In: *Proceedings of Animals in Science Conference: Perspectives on their Use, Care and Welfare* (Johnston NE, ed). Monash University, pp 131–48

Morton DB (1995b) The post-operative care of small experimental animals and the assessment of pain by score sheets. In: *Proceedings of Animals in Science Conference: Perspectives on their Use, Care and Welfare* (Johnston NE, ed). Monash University, pp 82–7

Morton DB (1997a) A scheme for the recognition and assessment of adverse effects. In *Animal Alternatives, Welfare and Ethics* (van Zutphen LFM, Balls M,eds). Amsterdam: Elsevier Science, pp 235–41

Morton DB (1997b) Ethical and refinement aspects of animal experimentation. In: *Veterinary Vaccinology* (Pastoret P-P, Blancou J, Vannier P, Verscheuren C, eds). Amsterdam: Elsevier Science, pp 763–85

Morton DB (1998a) The recognition of adverse effects on animals during experiments and its use in the implementation of refinement. *Proceedings of the Joint ANZCAART/NAEAC Conference on Ethical Approaches to Animal-Based Science*, Auckland, New Zealand, 19–20 September 1997. ANZCCART, PO Box 19, Glen Osmond, SA 5064, Australia, pp 61–7

Morton DB (1998b) The use of score sheets in the implementation of humane endpoints. *Proceedings of the Joint ANZCAART/NAEAC Conference on Ethical Approaches to Animal-Based Science*, Auckland, New Zealand 19–20 September 1997. ANZCCART, PO Box 19, Glen Osmond, SA 5064, Australia, pp 75–82

Morton DB (1998c) The importance of non-statistical design in refining animal experimentation. ANZCCART Facts Sheet. *ANZCCART News* **11**, No. 2, June 1998 Insert, ANZCCART, PO Box 19, Glen Osmond, SA 5064, Australia, p 12

Olfert ED (1995) Defining an acceptable endpoint in invasive experiments. *Animal Welfare Information Center Newsletter* **6**, 3–7

Olfert ED (1996) Considerations for defining an acceptable endpoint in toxicological experiments. *Lab Animal* **25**, 38–43

Russell WMS, Burch RL (1959) *The Principles of Humane Experimental Technique*. London: Methuen & Co [Special edition, 1992, Potters Bar: UFAW]

Soothill JS, Morton DB, Ahmad A (1992) The HID50 (hypothermia-inducing dose 50): an alternative to the LD50 for measurement of bacterial virulence. *International Journal of Experimental Pathology* **73**, 95–8

Ullman-Culleré MH, Foltz CJ (1999) Body condition scoring for assessing health status in mice. *Laboratory Animal Science* (in press)

van den Heuvel MJ, Clark DG, Fielder RJ, *et al*. (1990) The international validation of a fixed dose procedure as an alternative to the classical LD50 test. *Food Chemical Toxicology* **28**, 469–82

Wadham JJB (1996) *Recognition and Reduction of Adverse Effects in Research on Rodents* (PhD thesis). Birmingham: University of Birmingham