

Defining endpoints: the role of the animal care committee

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Summary

Although all individuals who use animals in research bear a responsibility to ensure that animal pain and suffering are minimized to the greatest extent possible, in the USA the ultimate responsibility rests with the Institutional Animal Care and Use Committee (IACUC). However, proposing refinements, and especially alternative endpoints and husbandry methods ('enrichments'), remain difficult areas for IACUCs. Refinements have received less attention than non-animal alternatives or methods for reducing the numbers of animals used, although emphasis now seems to be shifting. Nevertheless, there is presently little concrete information available to IACUCs about scientifically valid alternative endpoints. Establishing endpoints can therefore involve the committee, veterinary and animal care staff, and investigators in time consuming and expensive observations, and in institutions with large research programmes it is unrealistic to expect that this can be done for each new project. Increased support from funding agencies for pilot studies and refinement research in general (including improved housing methods) would be a welcome stimulus for promoting the search for more humane endpoints. Improved dissemination of information among institutions that have conducted refinement studies is also critical, and priority should be given to developing better methods for this information exchange.

The role of the animal care or animal ethics committee in defining endpoints is seemingly straightforward. In the USA, it is the responsibility of the committee to ensure that any proposed research is conducted in accordance with the principles of replacement, reduction, and refinement (Russell & Burch 1959). In practice, however, determining appropriate endpoints is probably the most challenging task facing such committees. Whilst general guidelines can be developed for certain types of procedures, in most cases the amount of pain and suffering experienced by the animal, as well as the experimentally and ethically acceptable cut-off point or points, must be evaluated on a study-by-study basis. In this paper, the role that the animal care committee plays with respect to refinement, and particularly in the establishment of humane endpoints is discussed. Since the comments are made specifically with reference to animal care

committees in the US, the system for oversight of animal research in the US will be described first.

Animal care committees in the United States

The system for oversight of laboratory animal use and care in the US is more dispersed than in many other countries. Any institution that engages in research, teaching or safety testing activities using warm-blooded animals must, under the Animal Welfare Act (CFR 1992) establish an oversight committee (referred to as the IACUC) to review proposed projects involving those animals. In addition, research, testing or teaching projects to be funded by federal agencies like the National Institutes of Health must be reviewed by the IACUC if any vertebrate animals are to be used (PHS 1996). Whilst in theory this means that many laboratory animals are not covered

under existing regulations or policies, in practice most institutions have chosen to oversee the use of all vertebrate animals, regardless of species or funding source.

Under the Animal Welfare Act, IACUCs must have at least three members, including a veterinarian, a scientist who uses animals in research, and a member who is not affiliated with the institution. This latter individual is intended to represent the 'general community's interest in the treatment of animals'. The guidelines used by the Public Health Service (PHS) further state that this individual not be a person who uses animals in biomedical research or teaching (ILAR 1996). The PHS also stipulates that the IACUC shall have at least five members, to include a non-scientist in addition to the members outlined above.

Among the functions of IACUCs are to ensure that there is an occupational health and safety programme in place for individuals who work with animals and that these same individuals are properly trained in the care and use of animals. The IACUC conducts semi-annual inspections of all animal facilities and animal study areas, and reviews the institution's overall animal care programme at least annually. The most critical role of the IACUC, however, is probably the review of proposed research, testing, and teaching activities, also known as protocol review. Protocols in the US are usually comprised of multiple related experiments, and the committee can approve them for a 3-year period with annual review (usually consisting of a determination that no changes have been made to the protocol as approved).

Whilst there are general guidelines and regulations covering IACUC activities, IACUCs in the US operate autonomously and tailor their committee composition and review procedures to most effectively address institutional needs and research programmes. There is, however, some national oversight of the care of animals and the conduct of institutional animal care programmes. The USDA inspects each institution that uses regulated animals semi-annually. In addition, certification is available to institutions on a voluntary basis through the Association for Assessment and

Accreditation of Laboratory Animal Care International (AAALAC). AAALAC conducts site visits of certified institutions every 3 years.

When Congress passed the Animal Welfare Act in 1966, it gave the US Department of Agriculture (USDA) discretion to determine which species of warm-blooded animals were to be overseen in research and should therefore be covered under the Act. The USDA has progressively increased the number of species covered. However, as of February 1999 birds and purpose-bred rats and mice are still excluded from coverage because there is insufficient funding for the enforcement effort required.

An exception to this sometimes occurs in the case of agricultural animals used for production-related research, which institutions may exclude from their oversight programme because the Animal Welfare Act and the Public Health Service Policy refer only to animals used in biomedical research. A voluntary guideline produced by a group of scientific societies, *The Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching* (FASS 1999), is used by many of the institutions that do provide oversight for these animals.

Protocol review

The overall responsibility of an IACUC is best summarized by the Three Rs—replacement, reduction and refinement (Russell & Burch 1959). Bearing these three principles in mind, the IACUC must ensure that animal suffering is minimized in a manner that is consistent with the achievement of the objectives of the proposed research. Because IACUCs operate autonomously in the US, there is no nationally standardized form used to obtain information about research and teaching projects, although the regulations and guidelines stipulate that protocols contain certain types of information. In general, committees require investigators to describe:

- the hypotheses, objectives, and significance of their research;
- the procedures that will be conducted on live animals;

- the rationalization for the numbers of animals to be used;
- the reasons for selecting the particular species of animal to be used;
- the methods for eliminating or minimizing pain or distress or a justification for not alleviating pain or distress;
- the training or expertise of investigators and staff in the proposed methods; and
- methods of euthanasia to be used, and the disposal of the animals at the end of the study.

Under the Animal Welfare Act regulations, IACUCs are also required to ensure that the investigator has conducted an adequate search for alternatives.

The ultimate decision of the IACUC regarding modification or disapproval of a particular protocol is final, i.e. the decision cannot be overridden at either the institutional or national level. However, there can be constraints, some obvious and some subtler, on the IACUC's latitude to modify or even reject particular protocols. One obvious one is when there are regulatory requirements that stipulate particular dose schedules or minimum numbers of animals, as may occur with toxicity testing. Another more subtle constraint involves the character of research programmes. Institutions, not IACUCs, determine institutional research priorities. And because research is increasingly costly, institutional priorities in turn are often driven by the priorities of funding agencies. In this sense, the decision about whether a particular line of research (although not a particular individual protocol) is scientifically meritorious has often already been made before the protocol even reaches the IACUC.

The US Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training (IRAC 1985) state that 'Procedures involving animals shall be designed and performed with due consideration of their relevance to human or animal health, the advancement of knowledge, or the good of society'. Driscoll and Bateson (1988) presented a decision-making cube in which the scientific merit of a study is one of three critical elements to be assessed in

arriving at a decision about whether or not to approve a particular protocol. However, by and large IACUCs in the US do not view it as their role to consider the scientific merit of a proposed research project in the sense described by Driscoll and Bateson. Since IACUCs are not constituted as scientific review bodies they rarely have the breadth of expertise necessary to conduct a full peer review. In addition, much (though by no means all) research has already been or will be reviewed by a funding agency peer review panel prior to being conducted. Prentice *et al.* (1992) argued that thorough review by the IACUC of hypotheses, experimental methods, statistical design, and other refinements in fact constitutes a merit review.

In the remainder of this paper, the IACUC's role in reviewing and recommending such refinements, particularly in the context of humane endpoints will be discussed.

Role of the IACUC

The IACUC has a special responsibility to scrutinize protocols that involve intense or prolonged unalleviated distress or pain especially carefully (Browder 1995, Olfert 1995). As a first step, it is helpful if each procedure to be performed on an animal can be categorized with respect to the procedure's potential to cause pain or distress. A number of different scales varying in complexity have been developed for this purpose (see Orleans 1993). Our IACUC uses a slightly modified version of the Canadian Council on Animal Care's scale (CCAC 1991), in which procedures are categorized according to four degrees of severity:

- **Category 1**—Little or no discomfort or distress (e.g. colony maintenance, blood sampling, physical examinations)
- **Category 2**—Minor stress or pain of short duration (e.g. minor procedures performed under anaesthesia)
- **Category 3**—Moderate to severe distress (e.g. major procedures under anaesthesia; prolonged restraint)
- **Category 4**—Severe pain near, at or above the pain tolerance threshold (e.g. highly

invasive surgery, death as an endpoint)

With this scale, the use of procedures falling into Category 4 (as well as Category 3 if the distress is unalleviated) would need to be particularly strongly justified scientifically, and possible alternatives thoroughly described and considered. If the distress is protracted or death or morbidity is the endpoint, the IACUC may need also to request information about the following:

- The expected time course for the animal.
- The amount of pain or distress that the animal might experience at each point during the time course.
- Whether pain or distress can be alleviated at any of the points during the time course without compromising the research.
- The specific criteria to be used for intervening, terminating the study, or euthanizing an animal.
- The frequency with which animals will be monitored at the different points during the time course.
- The training or experience of the personnel monitoring the animals in recognition of appropriate clinical signs.
- The rationale for the particular endpoint chosen, and reasons why earlier endpoints cannot be used.

There are already published guidelines available for some procedures that outline the time course of responses and recommend appropriate endpoints (e.g. for monoclonal antibody ascites production). Where such guidelines are not available, previous studies of the particular model under investigation can be used in determining the time course and the probable pain or suffering associated with the procedure, although deciding whether an earlier endpoint can be used can still prove problematical (see below). In some cases there are no preliminary studies or established guidelines, and cooperation and dialogue between the IACUC, the investigator, and the veterinary staff will be critical in arriving at the most humane and scientifically appropriate endpoints.

Establishing endpoints

Olfert (1995) outlined the steps that need to be taken when attempting to select an earlier endpoint. The first step is to objectively outline the signs and symptoms of pain and distress. The second is to determine which signs and symptoms are most significant, and the last is to ensure that the measurements are scientifically acceptable. Since Morton and Griffiths published their system for scoring pain and distress in laboratory animals in 1985, there has been increasing interest in developing similar score sheets for animals undergoing specific experimental procedures (see Olfert 1995, Morton 1999), with a view towards determining less severe endpoints that reliably predict definitive endpoints. As the papers in the current volume demonstrate, this assessment has to be based on careful and often detailed observation of the animals during the course of the procedure. This may mean that a preliminary study needs to be conducted, and/or that the IACUC (or someone delegated by the IACUC) may need to be involved in monitoring the animals during the initial study.

Sometimes it is impossible to predict the specific effects of particular manipulations in advance. An example would be the production of new lines of transgenic animals using the microinjection method. With this method, it is difficult to control where the introduced gene is inserted, the number of copies of the gene inserted, and the timing of expression of the inserted gene. Animals expressing the transgene can therefore experience a wide range of defects that may vary from mild to severe, and that may only become apparent at certain stages of development or not even until the second generation (Mench 1999). Some defects, like subtle behavioural deficits or a change in the animal's ability to cope with stressors, may be difficult to detect using normally accepted clinical signs, but they may nevertheless have negative consequences for the animal's welfare. In these situations an active monitoring programme will need to be maintained, and the IACUC will need to work with the investigator on an assessment scheme for the animals to determine appro-

appropriate endpoints. The IACUC may also wish to have the investigator submit periodic reports of the percentage of animals expressing the transgene, the types and degrees of severity of defects observed, the numbers of animals affected, the criteria used for euthanasia, and the numbers of animals that had to be euthanized.

Although studies in which death is the endpoint have generated the most concern (Browder 1995), studies that do not result in the animal's death but do have the potential to cause prolonged distress can prove equally, if not more, problematical for the IACUC. A case in point is the use of rhesus monkeys in research on mapping of the visual or auditory cortex. A standard protocol for these monkeys involves many months of training on extremely complex visual or auditory tasks, followed by lengthy testing involving hundreds of recordings of single cells in the cortex through implanted electrodes. The monkeys are fluid restricted, chaired during task performance, and given a water or juice reward when they perform the task correctly.

These studies raise a number of difficult questions. Are there alternatives to the use of fluid restriction? If water restriction is to be used, should there be a limit on the amount of water restriction, and if so, how can that limit be determined? Should 'break' periods be provided where the monkeys are returned to *ad libitum* water? Should there be a maximum number of hours that the monkey is asked to work in a day, or a maximum number of days worked in a week, or a month, or a year? Should criteria be set to remove monkeys from the study who appear to be having difficulty adapting to the water restriction regimen or the testing conditions, and if so, what should the criteria be? When a particular monkey has successfully adapted to the study conditions and the investigator has taken all necessary recordings at the implant site, should that same monkey be used for another study requiring a new implant, or should a new monkey be used? If the former, how many implants should be allowed?

Long-term experiments of this type require the committee to address a life plan for the animal that involves establishing not just

one endpoint, but endpoints within endpoints. In the case of the macaques, our IACUC has worked with the veterinary staff to conduct extensive behavioural and physiological monitoring to determine valid criteria for decision making. We established daily and weekly water restriction limits based on regular assessment of food intake, body weight loss, urine specific gravity, serum sodium level, blood urea nitrogen and creatinine levels, urine osmolality, behavioural signs of severe agitation or depression, skin turgor, and the quantity and quality of the monkey's stools. A 2-week programme was also developed to condition the monkeys to the water restriction regimen prior to being placed on the study; monkeys that show difficulty in adapting are not used in the study. We are currently assisting the investigators to develop assessment criteria to determine, before the electrodes are implanted, which monkeys will be most likely to adapt well to the test conditions and also be highly motivated to perform, perhaps even at a reduced level of restriction. Lastly, we are examining the records of performance of each monkey to see if limits can or need to be set on the amount of time spent working.

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