ELSEVIER

Guidelines for the Use of Animals

Guidelines for the treatment of animals in behavioural research and teaching

Behavioural studies are of great importance in increasing our understanding and appreciation of animals. In addition to providing knowledge about the diversity and complexity of behaviour in nature, such studies also provide information crucial to improvements in the welfare of animals maintained in laboratories, agricultural settings and zoos, and as companion animals. The use of animals in behavioural research and teaching does, however, raise important ethical issues. While many behavioural studies are noninvasive and involve only observations of animals in their natural habitat, some research questions cannot be addressed without manipulation of animals. Studies of captive animals necessarily involve keeping animals in confinement, while at times studies involving wild animals require that provision is made for trapping and subsequent release of the animals. Consideration has to be given to appropriate marking techniques to allow individuals to be distinguished, and manipulative procedures and surgery may be necessary to achieve the aims of the research. Studies of free-living animals in their natural habitats can cause disruption to the animals' population or the wider ecosystem, particularly if feeding, capture, marking or experimental procedures are involved. While the furthering of scientific knowledge is a proper aim and may itself advance an awareness of human responsibility towards animal life, the investigator must always weigh the potential gain in knowledge against any adverse consequences for the individual animals, populations under study, and the wider ecosystem. This is equally true for the evaluation of animal use in animal behaviour teaching activities. In fact, animal behaviour courses provide an excellent opportunity to introduce students to the ethical obligations a researcher accepts when animals are studied.

To help both researchers and teachers make what are sometimes difficult ethical judgements about the procedures involved in the study of animals, the Association for the Study of Animal Behaviour and the Animal Behavior Society have formed Ethical and Animal Care Committees, respectively. These committees jointly produced the following guidelines for the use of all those who are engaged in behavioural research and teaching activities involving vertebrate and invertebrate animals. These guidelines are general in scope, since the diversity of species and the study techniques used in behavioural research preclude the inclusion of prescriptive standards for animal care and treatment, other than emphasizing the general principle that the best animal welfare is a prerequisite for the best science. A variety of sources give more details on the principles on which the guidelines are based (Hubrecht & Kirkwood 2010). The guidelines are used by the Editors of *Animal Behaviour*

in assessing the acceptability of submitted manuscripts. Submitted manuscripts may be rejected by an Editor, after consultation with the appropriate Ethical or Animal Care Committee, if the content is deemed to violate either the letter or the spirit of the guidelines. The ethical acceptability of manuscripts considered for publication in *Animal Behaviour* is weighed up as a cost–benefit analysis. Costs are considered to be costs to the animals (e.g. compromises of animal welfare, reduction of likely survival rates or reproductive success) or the environment, or reductions in the quality of science. Benefits are considered to be the value of the specific scientific insights sought to humans, other animals or the environment (i.e. whether the science is of good quality and addresses questions of importance). Any study that allows or precipitates great costs to animals for research must have both the highest potential benefits and the highest ethical justification. Great costs can be 'offset' in the cost–benefit analysis by achieving a high quality of research and/or answering very important questions. During ethical assessment of papers submitted to *Animal Behaviour*, the costs and benefits are weighed on a case-by-case basis to assess whether costs have been minimized, the benefits maximized, and whether the benefits outweighed the costs, before making a recommendation on publication. For this review process to function effectively it is vital that authors supply detailed information on the ethical treatment of their animals (see Guide for Authors, http://www.elsevier.com/wps/find/journaldescription.cws_home/622782/authorinstructions) providing details of the capture, care, marking, treatment and subsequent release or disposal of their study animals. This process uses the same logic that national, state or institutional ethical licensing bodies utilize (see below). But these guidelines act to supplement the legal requirements in the country and/or state or province in which the work is carried out. They should not be considered an imposition upon the scientific freedom of individual researchers, but rather as an aid to provide an ethical framework that each investigator may use in making and defending decisions related to animal welfare.

LEGISLATION

Investigators are accountable for the care and wellbeing of animals used in their research and teaching activities, and must therefore abide by the spirit as well as the letter of relevant legislation. It is their responsibility to acquire knowledge about local legislation. Appendix 1 lists sources of information relating to the legislation of several countries. Bayne et al. (2010) provide an

overview that includes additional countries. Investigators must familiarize themselves with legislation both on animal welfare and on threatened and endangered species, and conform with the spirit and letter of the laws. When submitting manuscripts to *Animal Behaviour*, all authors must verify that they have identified and adhered to the legal requirements of the country in which the study was conducted, and provide relevant permit numbers. Many nations and academic institutions require that experiments performed on captive animals or on wildlife that are manipulated in some way must first be reviewed and approved by an animal welfare, animal care and use, or ethics committee of the sponsoring institution (Jennings 1994; Hagelin et al. 2003). It is recommended that investigators from countries without any legal requirements or guidelines voluntarily refer to one or more of the documents in Appendix 1 for guidance. A manuscript based on institutional committee-approved research may still be referred by Editors or reviewers to the Ethical and Animal Care Committees of ASAB/ABS, if they feel the manuscript raises ethical concerns.

THE THREE R'S: REPLACEMENT, REDUCTION AND REFINEMENT

Much of the current recommendations and legislation for ensuring appropriate animal care and use are based on the three guiding principles of replacement, reduction and refinement (3Rs; Russell & Burch 1959; Buchanan-Smith et al. 2005; Manciocco et al. 2008; Vitale et al. 2008; Kilkenny et al. 2010; Richmond 2010). Replacement refers to efforts to replace animal subjects and models with nonanimal ones, such as tissue cultures or computer models, wherever it is possible to do so while still achieving the scientific objectives. Reduction means reducing the numbers of animals affected by the experiment to the lowest number of individuals necessary to achieve the aims of the experiment and statistical power. Experimental design and choice of statistics are critical to this. There will be occasions when it is possible to reduce the total number of animals used in an experiment, but only by increasing the degree or duration of discomfort for the fewer individuals that are used. In such cases, the investigator must find an ethical balance between the two principles, and decide which produces the least ethical harm. Refinement refers to efforts to design and conduct the study as carefully as possible to maximize the scientific benefit while minimizing suffering to the animals, for example by planning and implementation of humane endpoints in the event that problems arise (Richmond 1998; Rowan 1998). Research on wildlife often raises particular issues and difficulties, and recent advice on practice in this area can be found in Lane & McDonald (2010) and Inglis et al. (2010). The implementation of the 3Rs in behavioural research raises issues regarding the scientific reporting of studies, recently addressed by the National Centre for the Replacement, Refinement and of Reduction of Animals (Kilkenny et al. 2010).

CHOICE OF SPECIES AND NONANIMAL ALTERNATIVES

Investigators should choose species and strains for study that are appropriate and best suited for investigation of the questions posed. Choosing these requires knowledge of natural history, physiology and phylogenetic relationships. Knowledge of an individual animal's previous experience, such as whether or not it has spent a lifetime in captivity, is also important. Recent advances in genetic characterization of many laboratory animals may also allow the investigator to control for the effects of genotype on expected behavioural traits. The specialist characteristics and needs of some genetically altered strains must also be considered (Robinson et al. 2003); these are becoming increasingly common in behavioural studies and their welfare status and responses can

be considerably different to those of nonmodified animals (Alleva & Vitale 2000; Branchi et al. 2007). When research or teaching involves procedures or housing conditions that may cause pain, discomfort or stress to the animal, and when alternative species or strains can be used, the researcher should use the species or strain that is believed to be the least likely to experience pain or distress (OTA 1986). The fact that a species being studied is classified as 'vermin' in the country concerned does not free the researcher from normal obligations to the experimental animals. The majority of invertebrate species are usually excluded from legislation regulating scientific research on animals. This does not mean that they are all unable to experience pain, discomfort or stress, but knowledge is more limited than for vertebrate groups (Sherwin 2001; Gherardi 2009). Manuscripts involving research with invertebrates may still be reviewed by the Ethical and Animal Care Committees in light of the most current knowledge in this respect. Researchers using such species should seek expert advice and take any evidence on this matter relating to their species into account when designing experiments, and should endeavour to minimize potential harm wherever possible. Live animal subjects are generally essential in behavioural research, but nonanimal alternatives such as video records from previous work or computer simulations can sometimes be used (Smyth 1978; van Zutphen & Balls 1997). Material of this kind also exists or can be produced for teaching purposes and can sometimes be used instead of live animals to teach aspects of the behavioural sciences (van der Valk et al. 1999; Smith & Smith 2001).

NUMBER OF INDIVIDUALS

In compliance with the principles of replacement, reduction and refinement, any experiment should use the minimum number of animals necessary to test the hypotheses, without the loss of scientific rigour (Russell & Burch 1959; Still 1982; Festing et al. 2002). This should not only be applied to studies that involve procedures or treatments that may have a negative impact upon an animal or population, but should be adopted in the design of noninvasive experiments to ensure limited impact upon the subjects. Pilot studies, good experimental design and the use of statistical tests that enable several factors to be examined simultaneously are ways in which a researcher can reduce the number of animals used without compromising the research objectives (Hunt 1980; Still 1982; Dell et al. 2002; Nakagawa & Cuthill 2007). Statistical tests, such as power analyses of pilot data, can calculate the lowest number of animals needed to obtain meaningful scientific data (Kraemer & Theimann 1987; Cohen 1989). However, in the absence of pilot data, sample sizes should be based upon related published studies and the researcher should consider preliminary statistical analyses during the experiment to determine whether additional animals are required. Employing robust experimental design and data analysis are vital when determining the number of animals needed since surveys of published studies have concluded that fewer animals could have been used to obtain the same outcomes (Douglas et al. 1986; Kilkenny et al. 2009). It is equally important not to use so few animals that the research is invalid. Useful reference works are Morris (1999) and Ruxton & Colegrave (2006).

The use of genetically modified (GM) animals is increasing; however, careful consideration of their use is recommended. There may be underlying ethical and welfare problems associated with their use. These include the large numbers of animals used in the pre-experimental production phase and that the genetic modification may itself lead to a detrimental altered phenotype. There are published discussions of the pros and cons of using genetically altered animals (Hubrecht 1995; Boyd Group 1999; Wells et al. 2007).