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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

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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).

PROCEDURES

Investigators are encouraged to discuss with colleagues both the scientific value of their research proposals and possible animal welfare and ethical considerations. There are several models for evaluating animal research that can be of use when making ethical decisions (Bateson 1986; Orlans 1987; Shapiro & Field 1988; Donnelley & Nolan 1990; Porter 1992; de Cock Buning & Theune 1994; Fraser et al. 1997; Sandøe et al. 1997; Richmond 1998; Fraser 1999; Magalhães-Sant'Ana et al. 2009). If procedures used in research or teaching involve exposure to painful, stressful or noxious stimuli, whether through acts of commission or omission, the investigator must consider whether the knowledge that may be gained is justified. This will partly depend upon the goal of the research (e.g. research designed to enhance our understanding of animal welfare issues immediately may be judged differently to research designed for other purposes). Custom and practice, economic savings, convenience, or the fact that an animal might experience the same or similar stimuli in the wild are not adequate justifications. It should be borne in mind that the welfare costs of animal use may reflect not only the infliction of that which is unpleasant, but the denial of that which is pleasurable. There is a considerable amount of literature discussing the assessment of pain, suffering and wellbeing in both vertebrates and invertebrates (e.g. Morton & Griffiths 1985; AVMA 1987; Bateson 1991; NRC 1992; Broom & Johnson 1993; Flecknell 1994; Hubrecht & Kirkwood 2010; Morton 1998; USDA 1999; Flecknell & Waterman-Pearson 2000; Hellbrekers 2000; Sherwin 2001; Scott et al. 2003; Jordan 2005; Dawkins 2006; Jones & McGreevy 2010). Researchers are urged to consider the use of more refined procedures before using techniques that are likely to cause physical or psychological discomfort to the animal (Kreger 2000; Lloyd et al. 2008). When attempting to identify potential alternative procedures, the investigator will need to consider the pain- or distress-causing potential of all aspects or stages of the procedures in an experiment. The possible procedural substitutes or refinements for each may be very different. Pain or suffering should be minimized both in duration and magnitude as far as possible under the requirements of the experimental design. However, it should be borne in mind that studies of factors such as housing or management procedures that may induce states of stress, anxiety and fear can be a necessary part of research aimed at improving animal welfare or scientific validity. Attention should be paid to the provision of proper pre- and postprocedural care to minimize preparatory stress, and immediate and residual effects. When a study involves any procedure or condition likely to cause more than short-term, low-intensity pain, discomfort or distress, then appropriate anaesthesia, analgesia, tranquillization and/or adjunctive relief measures should be used, sufficient to prevent or alleviate the discomfort, unless this would jeopardize the aims of the study. Use of analgesics may also be appropriate after such procedures to minimize pain and distress (Flecknell 1985; Benson et al. 1990; Flecknell & Waterman-Pearson 2000; Coulter et al. 2009; Stokes et al. 2009; see also Ernst et al. 2006). Advice on the appropriate use of analgesics and anaesthetics to minimize discomfort and distress should be taken from trained veterinarians. In certain species, appropriate procedural training of animals can reduce the stress of some experimental procedures (see Reinhardt 1997; Grandin 2000; Conour et al. 2006). The investigator should keep in mind that many forms of discomfort or suffering can involve experiences other than nociception, such as nausea, pruritis, thermal stress, social isolation or fear (McMillan 2003). Many of these can be alleviated or prevented by medication, procedures or housing conditions that specifically target those experiences. Investigators should consult with experts for guidance on how to control or pre-empt suffering in its various forms.

The following more specific points may be of use.

Fieldwork

Field studies, involving either observations or experimental manipulations, are a potentially powerful means to investigate animal behaviour in the natural contexts in which it evolved. However, field studies are potentially disruptive to the subjects and the wider ecosystem (e.g. Nisbet 2000; de la Torre et al. 2000; Williams et al. 2002), and can interfere with the very qualities that field studies are best equipped to investigate, namely the natural character of behaviour (Martin & Bateson 1993). Therefore, for both scientific and ethical reasons, investigators studying free-living animals are expected to take precautions to minimize the imposition of fear, distress or lasting harm on individual animals, as well as minimizing the impacts of the study on the populations and ecosystems of which the individual animals are a part.

Specifically, investigators should weigh the potential gain in knowledge from field studies against the adverse consequences of disruption for the animals used as subjects and also for other animals and plants in the ecosystem. A key issue to take into account is that field studies, whether observational or experimental, may have impacts reaching far beyond the focal individuals under study. For example, 'by-catch' from trapping the focal individuals of the study often involves trapping a considerable number of nonfocal individuals in the process. It is the total impact on the ecosystem as a whole that should be balanced against the scientific gain, when evaluating the design of a proposed study.

A range of behavioural research techniques, including capture, handling and marking of wild animals, fitting with data logging or transmitting devices (e.g. radiotransmitters, geolocators), collection of physiological data (e.g. blood or tissue samples) or the experimental manipulations themselves, may have adverse consequences, such as a reduced probability of survival or reproduction (e.g. Parris & McCarthy 2001; McCarthy & Parris 2004; Drolet & Savard 2006; Knapp & Abarca 2009). Such impacts may be immediately apparent, or they may be delayed and thus potentially hidden from the experimenter (Putman 1995). Investigators should consider the effects of such interference, and select the least disruptive, as well as the least stressful, techniques available in the context of the study (Beausoleil et al. 2004). This may include considering minimally invasive or noninvasive techniques for monitoring physiology (Cooper 1998; Gedir 2001) and for identifying individuals, such as the use of phenotypic features (e.g. Scott 1978) or genetic markers extracted from faeces (e.g. Beebee & Rowe 2008), instead of invasive tissue sampling or marking.

Cuthill (1991) discusses the ethical issues associated with fieldwork, and recommends pilot investigations to evaluate potential negative impacts and follow-up monitoring to detect and minimize longer-term or delayed effects. For example, pilot studies may be used to determine the minimally effective doses of chemicals required for physiological manipulations under field conditions. Results from such pilot studies may (if favourable) be used to justify procedures that might otherwise be questioned on ethical grounds, by inclusion in the Methods section of submitted papers. Full details of the pilot studies should be made available for scrutiny by journal Editors and reviewers.

When an experimental protocol requires that animals be removed from the population either temporarily (e.g. for fitting a tag) or on a longer-term basis (e.g. as part of a mate removal experiment), investigators should ensure that suffering or discomfort is minimized not only for the removed individuals but also those dependent on them (e.g. dependent offspring or eggs). Removed individuals and their dependants should be housed and

cared for appropriately, their time in captivity minimized, and their safe replacement ensured.

Decisions about the welfare of individuals and the ethics of behavioural studies are particularly important when they involve rare and endangered species. Researchers planning research on species at risk of extinction, or translocations or reintroductions of animals as part of behavioural studies should first consult the current IUCN Guidelines and Policy Statements on Species-Related Issues (www.iucn.org).

Aggression, Predation and Intraspecific Killing

The fact that the agent causing harm may be another nonhuman animal does not free the experimenter from the normal obligations to experimental animals. Huntingford (1984), Elwood (1991), Bekoff (1993) and Bekoff & Jamieson (1996) discuss the ethical issues involved and suggest ways to minimize suffering, while Lind & Cresswell (2005) point out the problems in trying to devise experiments that determine the adaptive significance of antipredator behaviour, and show that many such studies are too simplistic and of questionable value. Note also that the conditions in which the animals are kept may influence rates of intraspecific aggression and killing (e.g. cleaning out cages at the wrong time can prompt infanticide in rodents; Burn & Mason 2008). Wherever possible, field studies of natural encounters should be used in preference to staged encounters. Where staged encounters are necessary, the use of models or video/film playback should be considered, the number of animals should be kept to the minimum needed to accomplish the experimental goals, and the experiments made as short as possible. Suffering can also be reduced by continuous observation with intervention to stop aggression at predefined levels, and by providing protective barriers and escape routes for the subjects.

Aversive Stimulation and Deprivation as Motivational Procedures

Aversive stimulation, deprivation or restriction of resources can cause pain or distress to animals. To minimize suffering, the investigator should determine whether there is an alternative reward strategy that could be used to motivate the animal in the study. If an alternative reward strategy is unavailable, or the effect of aversive stimulation is itself of interest (e.g. in studies of fear, stress or pain), investigators should ensure that the levels of restriction, deprivation or aversive stimulation used are no greater than necessary to achieve the goals of the experiment. Alternatives to aversive stimuli and deprivation strategies include the use of highly preferred foods and other rewards that may motivate even satiated animals (Reinhardt 1997; Laule 1999; Grandin 2000). Use of minimal aversive stimuli levels requires knowledge of the technical literature in the relevant area: quantitative studies of aversive stimulation are reviewed by Church (1971) and Rushen (1986), and the behaviour of satiated animals is considered by Morgan (1974). For some invertebrate groups the use of aversive stimuli/procedures should be considered carefully (Gherardi 2009), while for cephalopods there is evidence of the recognition of individual humans (Mather & Anderson 2007; Anderson et al. 2010). Before deciding in favour of aversion or deprivation, investigators should consider consulting with animal care staff, laboratory animal scientists and veterinary surgeons experienced in working with animals in research settings. Practical animal motivation and training is a highly specialized skill. The failure of positive reinforcement methods to motivate an animal may reflect only the strategy or tactic used, the skill of the research staff and level of rapport with the animal. It does not necessarily indicate that it would be impossible for a skilled trainer to motivate the animal successfully with positive reinforcement methods. Further comments on reducing distress caused by motivational procedures are to be found in Lea (1979) and Moran (1975). In practice, at all times positive training regimes should be considered before the use of aversive stimulation or deprivation. If this is not possible, then the use of aversive stimulation or deprivation must be strongly justified in submitted manuscripts.

Social Deprivation, Isolation and Crowding

Experimental designs that require keeping animals in overcrowded conditions, or that involve social disruption, deprivation or isolation, may be extremely stressful to the animals involved, and may adversely impact the behaviours being studied. Because the degree of stress experienced by the individual animal can vary with species, age, sex, reproductive condition, developmental history and social status, the natural social behaviour of the animals concerned and their previous social experience must be considered to minimize such stress (Shepherdson et al. 1998; Poole & Dawkins 1999).

Deleterious Conditions

Studies aimed at inducing deleterious conditions in animals are sometimes performed to gain scientific knowledge of value to human or animal problems. Such conditions include inducing disease, increasing parasite loads, and exposing animals to pesticides or other environmental stressors. Special care should be taken in studies involving genetically altered animals (e.g. transgenic animals, or those with induced mutations), because such modifications may compromise welfare even if this is not the primary goal of the modification. Standard welfare assessment procedures should be established and be in place before work commences (Westh Thon et al. 2010). Genetically altered animals should be checked or screened for possible welfare problems and their suitability as the most appropriate species/strain carefully considered. Studies inducing a deleterious condition in animals should address the possible treatment or alleviation of the condition induced. Animals exposed to deleterious conditions that might result in suffering or death should be monitored frequently using appropriately sensitive methods. Such methods may involve the use of behavioural indicators of harm, or tests that predict the development of serious states of harm, such as impending organ failure. Investigators are urged to seek the advice of experts on current methods for early detection of disease or harm resulting from deleterious conditions. In many cases, such tests can be performed on excreted body fluids. Whenever possible, considering the aims of the research, these animals should be treated or euthanized as soon as they show signs of distress. If the goals of the research allow it, the investigator should also consider experimental designs in which the deleterious condition is removed (e.g. removing rather than adding parasites as the experimental treatment) or in which naturally occurring instances of deleterious conditions are observed.

ENDANGERED SPECIES

All research on endangered or locally rare species must comply with relevant legislation and be coordinated with official agencies responsible for the conservation effort for the particular species or population under study. Legislation and sources of help in identifying endangered species can be found in Appendix 1. Members of threatened species should not be placed at risk except as part of a serious attempt at conservation. Observation alone can result in serious disturbance, including higher predation rates on nests of young, or their abandonment, and should be undertaken only after

careful consideration of techniques and of alternative species. Investigators should also consider further adverse consequences of their work, such as opening up remote areas for subsequent access or teaching techniques of anaesthesia and capture that might be misused (e.g. by poachers).

PROCUREMENT AND TRANSPORT OF ANIMALS

When it is necessary to procure animals either by purchase or by donation from outside sources, only reputable breeders and suppliers should be used (see Appendix 1). If animals are procured by capture in the wild, this must be done using methods that minimize pain, distress and suffering, and must comply with any relevant legislation. Investigators who purchase animals from local trappers should not encourage methods of trapping that cause suffering, or methods that involve killing many individuals to obtain a few live specimens. Procurement practices also should not create a local market for culling wildlife for profit. Individuals of endangered species or populations should not be taken from the wild unless they are part of an active conservation programme. The investigator should ensure that those responsible for handling purchased, donated or wild-caught animals en route to the research facilities are well qualified and experienced in the requirements of the species being transported, and that animals are provided with adequate food, water, ventilation, space and protection from wastes (IATA 2011). The stresses associated with transport should be identified and minimized. Animals should not be subjected to unduly stressful situations (e.g. excessive climatic conditions or sensory stimulation, prolonged food or water deprivation, aggression) during transport. Generally, only healthy animals that are able to withstand the rigours of transport should be transported. Young, dependent animals are not usually suitable for transport, but if transport is necessary, they should usually be transported only with the mother or equivalent. Special care, and additional regulations, may be relevant to the transport of pregnant animals. With particularly sensitive or social species, it may be necessary for a trained attendant or veterinarian to travel with the animal(s). Preconditioning animals to transport containers prior to shipment will reduce stress during loading and shipping. Furthermore, the investigator should carefully consider modes of transport, transport schedules (so as to reduce or eliminate layovers, unless rest periods are desirable) and shipping containers to ensure that they are suitable for the species being shipped. The relevant transport regulations for the species concerned (local, national and international) must be complied with.

HOUSING AND ANIMAL CARE

The researcher's responsibilities extend to the conditions under which the animals are kept when not being studied, as well as during study. Caging conditions and husbandry practices must meet, at the very least, minimal recommended requirements of the country in which the research is carried out (see Appendix 1). Although these publications provide general guidance, there is evidence that housing animals in larger or more enriched conditions than specified in these minimal requirements improves not only animal welfare (Newberry 1995; Kessler & Turner 1999; Mason et al. 2001; Olsson & Dahlborn 2002; Olsson et al. 2003; Sherwin & Olsson 2004) but also the quality of science, including behavioural studies (Poole 1997; Wurbel 2001, 2002; Sherwin 2004). Normal maintenance of captive animals should incorporate, as much as possible, aspects of the natural living conditions deemed important to maximizing welfare and survival. Consideration should be given to providing biologically relevant enrichment features such as natural material, refuges, perches, dust baths and water baths (Reinhardt & Reinhardt 2002), although it should be borne in mind that some enrichment features can sometimes create welfare problems of their own, for instance by increasing levels of competition and aggression (e.g. McGregor & Ayling 1990; Haemisch & Gartner 1994; Barnard et al. 1996). Companions should be provided for social animals where possible, providing that this does not lead to suffering or injury. Frequency of cage cleaning should represent a compromise between the level of cleanliness necessary to prevent diseases and the amount of stress imposed by frequent handling and exposure to unfamiliar surroundings, odours and bedding.

Standard housing and care regimes established for the commonly used laboratory animals are not necessarily suitable for wild animals or for individuals of wild species born in captivity. Special attention may be required to enhance the comfort and safety of these animals. Investigators may wish also to consult the most recent guidelines available from relevant taxon-oriented professional societies (Appendix 2).

The nature of human–animal interactions during routine care and experimentation should be considered by investigators. Depending upon species, rearing history and the nature of the interaction, animals may perceive humans as conspecifics, predators or symbionts (Estep & Hetts 1992). Special training of animal care personnel can help in implementing procedures that foster habituation of animals to caretakers and researchers and minimize stress. Stress can also be reduced by procedurally training animals to cooperate with handlers and experimenters during routine husbandry and experimental procedures (Biological Council 1992; Reinhardt 1997; Laule 1999).

FINAL DISPOSITION OF ANIMALS

When research projects or teaching exercises using captive animals are completed, it may sometimes be appropriate to distribute animals to colleagues for further study or breeding, if permitted by local legislation. However, if animals are distributed, care must be taken to ensure that the same animals are not used repeatedly in stressful or painful experiments, and that they continue to receive a high standard of care. Animals should never be subjected to major surgery more than once unless it is an unavoidable and justifiable element of a single experiment. Except as prohibited by national, federal, state, provincial or local laws, researchers may release field-trapped animals if this is practical and feasible, especially if it is critical to conservation efforts. However, the researcher should assess whether releases into the wild might be injurious or detrimental both to the released animal and to existing populations in the area. Because of potential impacts on the genetic structure of local populations in the area, animals should be released only at the site where they were trapped (unless conservation efforts dictate otherwise), and only when their ability to survive in nature has not been impaired and when they do not constitute a health or ecological hazard to existing populations. If animals are to be killed subsequent to a study, this should be done as humanely and painlessly as possible, and death should be confirmed before their bodies are destroyed. Where animals are killed, establishing that tissues or carcasses can be fully utilized, for example by other researchers, is in line with the principles set out in the 3Rs. Carcasses of wild animals killed with lethal anaesthetics or other toxic substances should be disposed of in a manner that would prevent scavengers from suffering secondary toxicity. Experts should be consulted for advice on methods of euthanasia that are appropriate for the particular species being used. Additional information on euthanasia methods can be found in the AVMA Guidelines on Euthanasia (AVMA 2007).

OBTAINING FURTHER INFORMATION

There are a number of organizations that provide publications and detailed information about the care and use of animals. Groups with an international focus that provide information on animal welfare include the International Union for the Conservation of Nature (IUCN 1995), the World Organisation for Animal Health (OIE, http://www.oie.int/), the International Fund for Animal Welfare (IFAW, http://www.ifaw.org/) and the European Food Safety Authority (EFSA, http://www.efsa.europa.eu/) which has published a series of guidelines and scientific opinions on the care and use of animals (http://www.efsa.europa.eu/en/ahawtopics/topic/animalwelfare.htm) particularly related to animals farmed for food production. National organizations providing information include the Canadian Council on Animal Care (Suite 1510–130 Albert Street, Ottawa, ON K1P 5G4, Canada, http://www.ccac.ca/), the Scientists' Center for Animal Welfare (7833 Walker Drive, Suite 410, Greenbelt, MD 20770, U.S.A., www.scaw.com), and the Universities Federation for Animal Welfare (The Old School, Brewhouse Lane, Wheathampstead, Hertfordshire AL4 8AN, U.K., http://www.ufaw.org.uk/). The Animal Welfare Information Center at the National Agricultural Library (10301 Baltimore Avenue, Room 410 Beltsville, MD 20705, U.S.A., http://www.nal.usda.gov/awic/) publishes a series of bibliographies on special topics, and can also provide individualized database searches for investigators on potential alternatives, including techniques for replacement with nonanimal models or alternative species, methods for reducing the total number of animals necessary to address the research question, and experimental refinements that can reduce pain and stress. The Animal Welfare Science Center (http://www.animalwelfare.net.au/) has produced some publications related to the care and use of animals as well as providing a list of links to other organizations.

The Internet provides a wealth of information on animal care and welfare issues. Many of these are government Web pages. A good starting place is the National Institutes of Health Office of Laboratory Animal Welfare site (http://grants.nih.gov/grants/olaw/olaw.htm), which contains considerable policy information and many links (e.g. http://grants.nih.gov/grants/olaw/links.htm), from which one can gain access to the 1996 Institute of Laboratory Animal Resources (ILAR) Guide for Care and Use of Laboratory Animals (published by the National Academies Press), as well as information on the IACUC Guidebook published by ARENA (Applied Research Ethics National Association). In some cases where newer editions are available, they should be preferentially consulted if possible. Another good source is the USDA/APHIS Animal Welfare Information Center site (http://www.aphis.usda.gov/animal_welfare/). The National Agricultural Law Center has an online animal welfare reading room (http://www.nationalaglawcenter.org/readingrooms/animalwelfare/). AAALAC International (Association for Assessment and Accreditation of Laboratory Animal Care) also has a home page (http://www.aaalac.org/) and offices in Europe and Southeast Asia. For information on organizations around the world involved in animal welfare, with an emphasis on animal protection, there is a directory hosted by WorldAnimal.Net (http://worldanimal.net/). In the U.K., the Department for Environment, Food and Rural Affairs (DEFRA) provides information about the Animal Welfare Act 2006 (http://www.defra.gov.uk/foodfarm/farmanimal/welfare/act/) while the Home Office provides information about the application of the Animals (Scientific Procedures) Act 1986 (http://www.homeoffice.gov.uk/science-research/animal-research/). Additional sources of information are NetVet (http://netvet.wustl.edu/), the National Academy of Sciences (http://www.nas.edu/) and the National Academies Press (http://www.nap.edu/). The Association of Zoos and Aquariums also has guidelines and advice on the husbandry of various zoo and wildlife species in captivity (http://www.aza.org/). ASAB/ABS does not necessarily endorse all the recommendations of these organizations, but we suggest that they make excellent sources of information from which to make challenging decisions regarding animal welfare.

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Appendix 1. Some Sources of Legislation and Regulations Regarding Animal Use and Procurement of Animals

International or Multinational

Bayne, K., Morris, T. H. & France, M. P. 2010. Legislation and oversight of the conduct of research using animals: a global overview. In: *The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals* (Ed. by R. Hubrecht & J. Kirkwood), pp. 107–123. Oxford: Wiley-Blackwell.
Convention on International Trade in Endangered Species of Wild Fauna and Flora. http://www.cites.org/.
International Air Transport Association (IATA). 2011. *Live Animal Regulations (LAR). The Global Standard for the Transportation of Live animals by Air*. http://www.iata.org/ps/publications/Pages/live-animals.aspx.

United Kingdom

Home Office Guidance on the operation of the UK legislation on animals used in research and codes of practice. http://www.homeoffice.gov.uk/science-research/animal-research/.
International Union for the Conservation of Nature, IUCN, The World Conservation Union Rue Mauverney 28, CH-1196 Gland, Switzerland. http://iucn.org/knowledge/publications_doc/.
Laboratory Animal Breeders Association of Great Britain. http://www.laba-uk.com/.
Radford, M. 2001. *Animal Welfare Law in Britain: Regulation and Responsibility*. Oxford: Oxford University Press.

United States of America

American Association for Laboratory Animal Science. http://www.aalas.org/Europe.
Code of Federal Regulations, *Title 9 (Animal and Animal Products), Subchapter A* (*Animal Welfare), Parts 1–3.* Available from: the Office of the Federal Register, National Archives and Records Administration, U.S. Government Printing Office, 732 N. Capitol Street, NW, Washington, D.C. 20401: http://www.gpoaccess.gov/cfr/index.html and APHIS, U.S.D.A., Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782. http://www.aphis.usda.gov/animal_welfare/index.shtml.
Code of Federal Regulations, *Title 50 (Wildlife and Fisheries), Chapter 1* (Bureau of Sport Fisheries and Wildlife Service, Fish and Wildlife Service, Department of Interior). Washington, D.C.: U.S. Government Printing Office. www.fws.gov.
FELASA, the Federation of European Laboratory Animal Science Associations. http://www.felasa.eu/.
Guide Development Committee 1988. *Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching*. Washington, D.C.: Consortium for Developing a Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching. Available from: Association Headquarters, 309 West Clark Street, Champaign, IL 61820.
NRC (National Research Council) 2010. *Guide for the Care and Use of Laboratory Animals. Committee for the Update of the Guide for the Care and Use of Animals.* National Research Council, The National Academies Press, Washington, D.C. http://dels.nas.edu/Report/Guide-Care/12910.
Office for Endangered Species, U.S. Department of Interior, Fish and Wildlife Service, Room 430, 4401 N Fairfax Drive, Arlington, VA 22203.

PHS (Public Health Service) 1986. *Public Health Service Policy on Humane Care and Use of Laboratory Animals*. Washington, D.C.: U.S. Department of Health and Human Services. Available from: Office for Protection from Research Risks, Building 31, Room 4809, NIH, Bethesda, MD 20892.
U.S. Department of Agriculture (USDA), Animal and Plant Inspection Service (APHIS). *Animal Welfare Electronic Freedom of Information Requests – Annual Reports*. (Searchable database of licensed animal breeders: 'View AWA Inspection Reports'.) http://www.aphis.usda.gov/animal_welfare/efoia/7023.shtml.

Europe

Council of Europe. European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes http://conventions.coe.int/Treaty/EN/Treaties/Html/123.htm.
Council of Europe 2006. Fourth multilateral consultation of parties to the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (ETS 123) https://wcd.coe.int/ViewDoc.jsp?id=1097275&Site=CM&BackColorInternet=9999CC&BackColorIntranet=FFBB55&BackColorLogged=FFAC75#RelatedDocuments.
European Commission 2007. Commission recommendations of 18 June 2007 on guidelines for the accommodation and care of animals used for experimental and other scientific purposes. Annex II to European Council Directive 86/609. See 2007/526/EC. http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2007:197:0001:0089:EN:PDF.
European Union. http://europa.eu/index_en.htm.

Canada

Canadian Council on Animal Care 1993. *Guide to the Care and Use of Experimental Animals. Vols 1 and 2*. Ottawa: Canadian Council on Animal Care. http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/gublurb.htm.
Canadian Council on Animal Care 2003. *Guidelines on the Care and Use of Wildlife.* Ottawa: Canadian Council on Animal Care. http://www.ccac.ca/Documents/Standards/Guidelines/Wildlife.pdf.
Committee on the Status of Endangered Wildlife in Canada, Canadian Wildlife Service, Environment Canada, Ottawa, ON K1A 0E7.
The Canadian Association for Laboratory Animal Science. http://www.calas-acsal.org/.

Australia

Commonwealth of Australia 2005. *Australian Animal Welfare Strategy.* Department of Agriculture, Fisheries and Forestry. Canberra, Australia. http://www.latrobe.edu.au/research-services/assets/downloads/Animal_Welfare_Strategy_2005.pdf.
National Health and Medical Research Council 2004. *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes*. Australian Government. http://www.nhmrc.gov.au/publications/synopses/ea16syn.htm.

New Zealand

Ministry of Agriculture and Forestry, New Zealand 1999. Animal Welfare Act 1999. Public Act 1999, Number 142. http://www.legislation.govt.nz/act/public/1999/0142/latest/DLM49664.html.
Ministry of Agriculture and Forestry, New Zealand 2006. *Guide to the Preparation of Codes of Ethical Conduct*. http://www.biosecurity.govt.nz/files/regs/animal-welfare/pubs/naeac/naeaccec.pdf.

Appendix 2. Taxon-oriented Societies with Ethical or Animal Welfare Guidelines

American Society of Mammalogists:
http://www.mammalogy.org/committees/index.asp.

Ornithological Council:
http://www.nmnh.si.edu/BIRDNET/GuideToUse/.

American Fisheries Society: http://www.fisheries.org/html/Public_Affairs/Sound_Science/Guidelines2004.shtml.

American Society of Ichthyologists and Herpetologists (ASIH), the Herpetologists' League (HL) and the Society for the Study of Amphibians and Reptiles (SSAR):
http://www.asih.org/files/hacc-final.pdf.

American Society of Ichthyologists and Herpetologists (ASIH), the American Fisheries Society (AFS) and the American Institute of Fisheries Research Biologists (AIFRB):
http://www.asih.org/files/fish%20guidelines.doc.

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