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Guidelines for the ethical use of animals in applied ethology studies

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Abstract

Applied ethology has a continuing interest in the promotion of animal welfare and the ethical treatment of animals used in research. However, in contrast to some other fields involving animal research, there are currently no guidelines written specifically for those engaged in applied ethology studies. We aim here, to provide members of the profession with a basis for structured self-evaluation of the ethical nature of their work, and to serve as inspiration for those planning research involving the use of animals. The first three sections of this document discuss the background to why ethical guidelines are needed in applied ethology studies and the relation between these guidelines and legislation. In the first section, we briefly discuss the relevant ethical principles and decision models. The main body of the guidelines then discuss how 'costs' to the animals in applied ethology research can be minimised (using the principles of replacement, reduction and refinement) and 'benefits' maximised. An earlier version of this manuscript was presented to the Annual General Meeting of the International Society of Applied Ethology, which accepted this as the basis of ethical review for papers presented at their International Congresses.

Keywords: Applied ethology; Behaviour; Ethics; Guidelines; Welfare

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1. Background

Almost all applied ethologists use animals for research or educational purposes. Many are involved directly in studying animal welfare and ethics, and some are involved in committee work or legislative procedure related to animal welfare and ethics. As a result, an area of applied ethology of continuing interest is the promotion of the welfare and ethical treatment of animals used in research. However, in contrast to some other fields involving animal research, there are currently no guidelines written specifically for those engaged in applied ethology studies. Moreover, some countries have little legislation and documented standards for guidance. Therefore, there is a need for a set of written guidelines specific for applied ethology. It is our aim that these guidelines will provide members of the profession with a basis for structured self-evaluation of the ethical nature of their work, and will serve as inspiration for those planning research involving the use of animals.

Applied ethologists originate from many nations with very different cultures and belief systems. In addition, they conduct a wide variety of studies using disparate species in very different contexts. These guidelines have been written with these great diversities in mind and, as a consequence, are broad rather than specific. They have been written to increase awareness, encourage individual thought and stimulate discussion of the ethical issues surrounding applied animal behaviour research. The focus is therefore on the ethical principles and how to handle these, rather than a list of do's and don'ts applicable to all scenarios.

2. Introduction

The use of animals in research and education has attracted ethical concern for many years, most notably in toxicology and bio-medical studies. More recently, ethical concerns have been raised over less invasive studies such as animal behaviour research (Mench, 2000). This suggests a need for justification of the use of animals in behavioural research, and some guarantee that the research is conducted in an ethically acceptable manner (Driscoll and Bateson, 1988). Concerns about the use of animals in research are being voiced by both the scientific and lay communities, evident for instance by journals imposing ethical reviews of manuscripts, requirements for funding proposals to have statements that ethical guidelines will be adhered to, and the increasing public requirement for 'transparency' of research. To address these concerns before conducting behavioural research, the investigator should first assess whether the purpose of the experiment is of benefit and justifies the use of animals. If the purpose is found to be justified, the investigator should next consider what criteria must be met for the experiment to be acceptable. This process includes an assessment of the likely pain, distress and suffering an experiment might cause, and an evaluation of what level of suffering, etc. can be considered acceptable in that particular context. The investigator should be able to explain and justify his/her conclusions in order to demonstrate awareness of the ethical issues and facilitate dialogue between interested parties. Finally, a critical assessment of the experimental design will promote better quality of the research.

3. Legislation

It is recognised that many countries already have legislation regarding the use of animals in research. The guidelines presented in this paper are not intended to replace or subvert this legislation, and ethologists should conduct research according to both the spirit and letter of their local legislation. However, investigators should be aware that legislation may sometimes be considerably dated, or have been formulated for commercial circumstances, e.g. with consideration for economics and large-scale practicality, rather than for research. As a consequence, some legislated minimum standards of care may be less appropriate for smaller scale, applied animal behaviour studies. To ensure the highest welfare and ethical standards, investigators should remain appraised of current relevant literature. In recognition of the work of many applied ethologists, it is hoped that these guidelines will not only serve to promote, but to advance animal welfare and ethics, rather than simply meeting the requirements of current 'rules' of legislation which are often minimum standards.

4. Ethical principles and decision models

By definition, applied ethologists conduct research on animals, or have a vested interest in behavioural research. These guidelines are therefore written with an acceptance that animals can be 'used' for the betterment of human or non-human animal species. There are different ethical stand-points whereby the use of animals in research can be evaluated, and several models outlining the decision process relating to the ethical use of animals in research (e.g. Bateson, 1986; Porter, 1992; De Cock Buning and Theune, 1994). From a utilitarian stand-point, performing research involving the use of animals may be justified if certain criteria are met, such as

> Using animals for scientific purposes is only acceptable when the harm (physical or psychological) done to animals is outweighed by the benefits of the research.

To determine whether the benefits of research outweigh the costs, a cost:benefit analysis can be performed. The 'costs' are assessed in terms of the harm likely to be experienced by the animals used in the research, and the 'benefits' in terms of the gains to other animals (of the same or a different species), humans, or the environment. However, the principle also implies that the ethically acceptable option is the one that provides most benefits and involves the least costs. Therefore, when planning a study, the aim should not be to simply reduce costs to a level lower than the benefits, rather, the costs should be decreased as much as possible (see Section 5) and the benefits maximised as far as possible (see Section 6). We emphasise here that this cost:benefit analysis should include any distress or harm caused by housing (or other experimenter influence) prior to and subsequent to the experimental phase of the research.

Applying decision models to one's own research can be an enlightening exercise as it can help analyse the cost:benefit of the research, perhaps with a fresh, external perspective. It is worth considering discussing the scientific significance and ethical issues of proposed research with colleagues in different disciplines, or lay-persons; if these people cannot be convinced a study is worth undertaking, the investigator should look carefully at the

reasons she/he believes it should. Finally, any animal investigator should never forget to ask the absolute question—"Can I justify the use of animals in this research?"

5. Reducing the costs

A widely accepted method of reducing the costs associated with animal research is implementation of the three R's, i.e. replacement, reduction and refinement (Russell and Burch, 1959).

5.1. Replacement

Replacement means either that more sentient species should be replaced by less sentient ones (but see the Section 5.3 below for caveats about assessing sentience), or that animals should not be used at all if the same research or related training/education can be achieved in other ways. This may be difficult to achieve in animal behaviour studies, although model animals, video-recordings, computer simulations, etc. can be used in some circumstances, particularly as a method to reduce (see Section 5.2) the total numbers of animals used. It has been suggested (Christiansen and Sandoe, 2000) that 'replacement' in some cases can be achieved by using animals on farms, commercial establishments, during transport or in the field, rather than animals obtained specifically for the research. That a practice is standard in one context, for example, the use of certain types of housing, restraint, or management on commercial farms, does not necessarily mean that it is ethically justifiable to replicate it in the laboratory if this research can be conducted in situ.

5.2. Reduction

Reduction means using the minimum number of animals necessary to achieve the aims of the research. However, the investigator should not reduce the number to so few that the results become statistically invalid. There is a tendency to use fewer animals when research involves species that are exotic or expensive to maintain. This could lead to such studies lacking validity and therefore ethical acceptability. On the other hand, if using fewer animals of an exotic species for economic reasons does not compromise scientific validity, then smaller numbers of non-exotic species should also be used on ethical grounds. The number of animals used can be minimised by several means.

5.2.1. Previous work

If similar work has been conducted previously, this can be used to estimate the number of animals needed to produce a definite result, or the data may sometimes be included in meta-analyses. The applicability and validity of previously published research must be considered if it is to be used in this way.

5.2.2. Statistical methods

A reduction in the number of animals used can be achieved by applying good experimental design, appropriate sampling techniques and statistical procedures that

enable several factors to be analysed at the same time (Hunt, 1980; Still, 1982; McConway, 1992; Chiarotti and Puopolo, 2000). This will depend, to some extent, on the behaviour being studied; if the behaviour being recorded is highly variable in nature or occurrence, data must be collected from a larger number of animals to reduce the variance to an acceptable level. In cases where variance is known or can be predicted, tests for statistical power can be employed to predict the smallest number of animals needed to test hypotheses.

5.2.3. *Epidemiological approach*

Sometimes it may be possible to study the spontaneous occurrence of behaviours as they occur on farms, zoos, in the wild, etc. In some cases, these field studies can eliminate the need for additional animals for the purposes of research. Such studies can also help identify which parameters are most likely involved in a particular behaviour, and this information can be used to effectively reduce the number of animals used in any further experimental study.

5.3. *Refinement*

The object of refinement is to reduce to an absolute minimum, the pain, distress or suffering imposed on *every* individual animal used. The word 'animal' (see Appendix A) is generally taken to mean higher-order animals usually thought capable of feeling pain or experiencing suffering in other ways. It should be noted that Appendix A does not differentiate between any of the listed species in their potential for suffering. Therefore, it is unacceptable to substitute one species for another on the list, e.g. frogs for rats, unless there is good knowledge that the former has a lesser capacity for suffering and this will not invalidate the aims of the research.

5.3.1. *Choice of species*

In choosing the species for a study there are several '-isms' to avoid. Sizeism should be avoided; there is little evidence that smaller animals (at least within the vertebrates) are any less capable of suffering. Speciesism (between non-human animals) should also be avoided; this can occur because of the animals' physical appearance or the ecological niche of its wild counterparts. Thus, some animals might be incorrectly regarded as less capable of experiencing suffering because we find their appearance or behaviour unattractive, or because in the wild they are a pest species or live in an environment we consider undesirable, e.g. toads, rats, squid. In addition, human anthropocentricity means it is often very difficult for us to empathise with the sensory perceptions of different species (e.g. the visual perturbations caused by placing animals with ultraviolet sensitivity into environments without ultraviolet light), or the motivations of another species (e.g. the frustration of a hen about to lay an egg but unable to find a suitable nest-site). Differences between our own sensory perceptions and motivations, and those of the species to be used should also be considered in deciding whether it is appropriate to use the intended species in the intended conditions.

The species chosen for a research programme should be the most appropriate for the information that the investigator wishes to gain. The choice will usually require knowledge

of the problem to be investigated, the species' natural history and the animals' previous experience. Often, the aim of applied behaviour studies is to understand the responses of a particular species in a particular environment. If an inappropriate species is used, the research might therefore be invalid requiring it to be repeated with a more appropriate species, thus making the initial study *less* ethically acceptable.

5.3.2. Pain, suffering and distress

For any research programme that might involve pain, suffering or distress, the investigator should assess thoroughly whether the information gained can be justified and if non-animal alternatives (e.g. models, video-playback, computer simulations) might be used. Pain, suffering or distress should be minimised to the greatest possible extent both in magnitude and duration, but without jeopardising the aims of the experiment. Some species appear to be less responsive to painful or stressful stimuli, however, this should not necessarily be taken as indicating that these species are more tolerant or do not experience pain and suffering. Animals might have evolved responses that mask evidence of pain or injury, presumably to avoid being targeted by predators.

In research involving surgery, a high standard of pre- and post-operative care must be implemented to reduce adverse effects both before and after the operation. Any procedure likely to cause pain should only be performed after adequate anaesthesia and with appropriate analgesia, unless either of these is contra-indicated by the experimental aims.

The use of neuromuscular blocking agents alone (i.e. causing the animal to become unable to move but without reducing its sensibility to pain) is generally unacceptable.

It should be considered that all higher-order animals (see Appendix A) have the capacity to experience pain and are capable of experiencing suffering of one kind or another. This will depend on many factors such as the species, age, sex, reproductive condition, social status, individual experience, perceptions, motivations and natural behaviour of the animal. The possibility that invertebrates such as spiders might experience pain or an analogous sensation (reviewed by Sherwin, 2001) should also be considered.

5.3.3. Housing

Standard housing of animals in farms, zoos and laboratories is often minimalist and designed primarily for the convenience of humans. This can result in the animals exhibiting behavioural or physiological responses indicative of reduced welfare, although it is difficult to assess how great this impact is. Several publications have ranked the severity of procedures conducted on animals in research (e.g. Morton and Griffiths, 1985; Bateson, 1991) and some define a category that includes procedures/studies that are thought unlikely to cause suffering. However, because housing animals under standard conditions is in itself likely to cause a degree of suffering even before any experimental procedures have been conducted, such a category might be overly optimistic. This means that in all circumstances, investigators should be able to ethically justify why an animal is being housed and/or why it is housed under particular circumstances, even if the research does not involve a procedure that causes overt pain or distress. To provide suitable housing and husbandry, investigators should consider both the quantity and quality of space they provide for their animals, and remain appraised of current relevant literature.

5.3.4. Identification of animals

It is often necessary to individually identify animals. There are many methods of achieving this. Wherever possible, non-invasive methods should be used, although these tend to be more short-term and might require repeated re-application thus potentially causing further distress to animals. Invasive methods that cause minimal pain and distress (e.g. ear-tags, wing-tags) are acceptable if they are in accordance with the aims of the study. The size of the identification device or marking method relative to the body-size of the animal should be considered, as well as any effects this might have on behaviour or possible suffering during and subsequent to attachment/implantation. Mutilatory forms of identification (e.g. toe-amputation), or those which injure substantial amounts of tissue should be assumed to cause substantial acute and perhaps chronic pain, and would therefore generally be considered unacceptable.

5.4. Other "standard practices" used in husbandry

A variety of practices that are likely to cause pain, distress or suffering are conducted routinely upon animals on farms, in laboratories, or other commercial establishments, e.g. beak-trimming, castration, chronic food deprivation, social isolation, etc. The fact that these practices are performed routinely elsewhere does not mean they should be placed above ethical scrutiny if they are performed on animals in a research study. Indeed, many of these practices can be considered unnecessary for animals in research, so long as their omission does not contravene the validity of the study or its aims.

5.4.1. Presence of experimenters and handling

The presence of humans can have a considerable effect on the behaviour of animals. This presence may or may not cause distress to the animals, but in either case, if this interferes with the aims of the study it reduces the validity of the research and therefore lowers the ethical acceptability. Investigators should consider the use of remote monitoring (e.g. video), or habituating the animals to the presence of humans. It should also be remembered that 'blind' studies in which the observer has no knowledge of which treatment the animal has been subjected to, reduces the likely influence of the observer and increases the validity of the research. The way in which animals are handled can have a substantial effect on their behaviour and welfare in both the short- and long-term. Poor handling can cause acute negative responses and can result in learned aversion to subsequent handling; potentially, this can invalidate the research. Investigators should familiarise themselves with the appropriate handling methods for the animals to be used.

5.4.2. Duration of the study

In applied behaviour studies, the end-point of a study is often relatively easy to decide. For example, a study on the behavioural responses of laying hens in a novel housing system in the UK might extend for 62 weeks because this is the average duration that hens are housed on farms there. Data collected beyond 62 weeks might be considered irrelevant and therefore less ethically acceptable. On the other hand, if the work were conducted in a country where hens were routinely housed for a longer period on farms in that country, it could be less ethically acceptable to terminate the study before the end of this period.

5.4.3. Final disposal and euthanasia

At the end of the study, investigators should consider alternatives to immediate euthanasia of the animals. There are sometimes good reasons for using animals in other studies (e.g. the animals are used to being handled, familiar with the environment or procedure), but care must be taken to ensure the animals are not used repeatedly in stressful or painful experiments. Livestock might be placed onto farms but the investigator should consider the likely responses of the animals to the change of social and physical environment, and the legal, ownership and disease consequences. Similarly, some species might be placed into private homes or sanctuaries.

Field-caught animals may be placed in zoos or reserves to reduce the need for further capture of wild animals, but again, the investigator should consider the likely responses of the animals to the change of social and physical environment, and the legal, ownership and disease consequences. Alternatively, field-caught animals may be returned to the place of capture if their ability to survive has not been impaired and release does not constitute a health or ecological hazard to existing populations.

The manner in which animals are euthanased is a significant component of the ethical acceptability of a research programme. In applied ethology studies, the method of the animals' killing will often not be under the control of the investigator, e.g. animals on farms will usually remain on the farm and will be slaughtered commercially. If the investigator has control over the method of euthanasia, factors to be considered are the likely duration of pain and distress caused by the method, and any handling the method requires. There is evidence that some methods of killing are less appropriate than others, despite their common use and approval by many legislative agencies. Investigators should read Appendix B in this regard. It should be remembered that methods of killing might be approved by legislation because of practicality and economic issues, rather than animal welfare. Death of the animal should be confirmed before the body is discarded.

5.4.4. Procedures

5.4.4.1. End-point of a procedure. Deciding on the end-point of a procedure, especially when this involves obvious pain, distress or suffering, is critical for the welfare of the animal and thus the ethical justification for the study. Investigators should consider choosing flexible end-points, e.g. in studies of aggressive or agonistic encounters, behavioural indicators of an animal accepting defeat are likely to cause less distress than encounters which have a fixed duration of interaction arbitrarily decided upon. Except under very exceptional circumstances, death as an end-point (i.e. the research requires animals to die) is unacceptable.

5.4.4.2. Aversive stimuli. Animals are sometimes deliberately exposed to aversive stimuli (e.g. electric shock, fear-inducing stimuli, predator–prey interactions, intra-specific competition, infanticide). If this is essential, it should be minimised in both severity and duration in accordance with achieving the aims of the experiment. The animals' perceptual and behavioural characteristics, age, experience, etc. should be considered in planning the study. Investigators should monitor such studies frequently, or preferably, constantly. At a pre-determined point, intervention should occur; the animal should be

removed from the study and given appropriate treatment or euthanasia. Barriers or escape routes should be provided for the animal to avoid the aversive stimulus where this is in accordance with achieving the aims of the experiment. Investigators should be aware of indicators of extreme fear, e.g. learned helplessness, and that some species may sometimes appear to become totally unresponsive even though they are aware and cognisant of their surroundings. Field studies should be considered as an alternative method of investigation.

5.4.4.3. Deprivation. Animals are sometimes deprived of various resources for a variety of reasons. These resources can be of various types, e.g. social contact, straw, perches, food, water, suitable light. If deprivation is essential, this should be minimised in both severity and duration in accordance with achieving the aims of the experiment. Food is sometimes withdrawn to motivate animals to perform a particular task, however, the use of highly attractive foods or other rewards is often a more acceptable alternative (there is also evidence that food deprivation can interfere with some learning tasks (e.g. Nicol and Pope, 1993). To avoid subjecting an animal to chronic hunger, it may be preferable to deprive it of food for a pre-determined period of time before testing, rather than attempting to achieve and maintain an arbitrarily specified target bodyweight. The method ultimately chosen will depend on the questions being asked and the protocol used to answer those questions.

5.4.4.4. Adverse conditions. Studies aimed at determining the effects of adverse conditions on animals are sometimes conducted to gain knowledge of applied problems, e.g. parasite loads, pesticides or homeostatic challenges. These conditions (e.g. parasite load) as well as the procedures used to induce them (e.g. gavage with oocysts) may cause suffering and should be minimised in both severity and duration in accordance with achieving the aims of the experiment. Investigators should plan frequent or constant monitoring of such studies, and appropriate intervention at a pre-determined end-point with appropriate care or euthanasia of the animals. Investigators should also consider experimental designs that allow removal of the adverse condition rather than its addition (e.g. the use of a novel insecticide on a population of sheep for which it is known that ecto-parasite burdens are already high), or naturally occurring instances of the adverse conditions.

5.4.4.5. Isolation and crowding. Many applied behaviour studies investigate the effects of isolation or crowded conditions that are used routinely on farms and in laboratories. It should be realised that although such housing might be considered standard in some contexts, these systems may be extremely stressful to animals (see Section 5.3.3 above). The degree of stress experienced will be markedly influenced by the species, age, sex, reproductive condition, social status, individual experience and natural behaviour of the animal. These factors should all be considered in the experimental design in order to minimise the stress likely to be experienced by the animal.

6. Increasing the benefits

As stated previously, the benefits of any proposed research should be made as great as possible. These can be maximised in several ways.

6.1. Achieving the aims

The aims of the research should be achievable. This can be ensured by closely examining the aims and determining if the appropriate animals, equipment, housing and trained personnel are all of adequate standard and are available for the duration of the study.

6.2. Significance of the aims

The aims can be of various forms, for instance involving health or welfare of humans or non-human animals, economic gains for livestock production, conservation, pest control or fundamental knowledge. In applied behaviour studies it is often possible to quantify and state the likely benefits resulting from research, and therefore the significance of the aims. For example, a researcher studying feather-pecking might be able to identify the aims of determining the average incidence of hens pecked, the average number of injuries, mortality rates and the cost of increased food consumption as the animals attempt to maintain their body temperature. Such information helps indicate the severity and extent of the problem being addressed, and therefore the likely significance of the findings. Although it may be more difficult to state the likely benefits resulting from fundamental research and harder to predict what the potential gain of the knowledge could be, fundamental research may provide essential information and possibly even support progress in the applied field.

6.3. Researching previous work

Investigators should thoroughly familiarise themselves with previously published relevant literature. This avoids unnecessarily duplicating research (assuming previous work was done correctly) although duplication may be required in pilot studies of a novel method. It will also be possible to gauge the variability of responses and ensure the experimental design is optimised to achieve the aims of the study by using the least number of animals.

6.4. Reporting of the study

A fundamental component of the ethical justification of animal behaviour research is the communication of results. The investigator has an ethical obligation to attempt to publish the results as completely, widely and as accurately as possible. Doing this decreases the probability of more animals being used in unnecessary duplicate studies to generate similar, redundant data. Widespread (global) communication of results is a ‘benefit’ factor in many models of the ethical assessment of animals and thus communication of results increases the benefits of the work. To demonstrate that an ethical assessment has been made, the ethical justification for choice of research and experimental design can be included. This will promote understanding and communication concerning the ethical issues and dilemmas in research involving animals (Christiansen and Sandoe, 2000).

7. Field experiments

Investigators conducting field experiments of applied animal behaviour should consider the ethical issues discussed above, and in addition, the impact of their work on other populations of animals and ecosystems. Methods of marking, the taking of physiological samples, capture, continuous observation, etc., might all influence an animal's ability to survive both at the time of observation and in the future. The welfare of other animals dependent on the subject (e.g. offspring) should also be considered. Cuthill (1991) and Kirkwood and Sainsbury (1996) discuss ethical issues associated with conducting field experiments.

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Appendix A. Higher-order animals

Higher-order animals include all vertebrates and of the invertebrates, some members of the phylum Mollusca (e.g. octopus, squid) and some members of the phylum Arthropoda (crab, lobster, crayfish). Higher-order animals are also considered to include mammalian foetuses during the last half of pregnancy, un-hatched young of the species stated above during the last half of their development in the egg, and marsupial pouch young. This is because it is thought likely that animals in these stages of development might be able to experience pain and suffering. (This classification of higher-order animals is adapted from New Zealand law which regulates the scientific use of animals, Animal Welfare Act, 1999.)

Appendix B. Comments on some commonly used methods of killing

Applied ethologists often conduct research with farm or laboratory species. When commercially reared, these species are often killed in great numbers and methods are used which take into account factors such as cost, speed of throughput, and practicality; the welfare of the animals may be given a lower priority than would be otherwise. When these species are killed after behavioural studies, the numbers involved are usually considerably lower than after rearing on farms or in commercial laboratories, and the animals are often very accustomed to being handled by humans. This makes alternative methods of euthanasia more practical and economically justifiable—or alternatively, allows more time to correctly administer the method of killing. A review of the literature reveals that some legally accepted methods of killing used commercially might be associated with

welfare concerns that appear to be widely unknown or perhaps ignored (see below), making these less ethically appropriate for use on animals used in small-scale research studies. We should remember that the term 'euthanasia' generally refers to 'an easy and painless death' or 'the killing of an animal with a minimum of physical and mental suffering, depending on the species' (Close et al., 1996), suggesting that legally accepted methods might not always be described as 'euthanasia'. Investigators are strongly encouraged to read Close et al. (1996) and the American Veterinary Medical Association (AVMA) Panel on Euthanasia (2000).

B.1. Decapitation and cervical dislocation

There is evidence that decapitation and cervical dislocation may not render an animal immediately unconscious. In rats, normal brain electrical activity indicative of consciousness can persist for 30 s after decapitation, and visual responses can be evoked from hens for 30 s after decapitation (Gregory and Wotton, 1986; see also Anon, 2000; Holson, 1992). Reptilian brain metabolism can function at low respiration and heart rates, and Warwick (1986) cites evidence that the heads of snakes will respond to approach for up to 59 min after decapitation. Cervical dislocation must achieve severance of both the spinal cord from the brain (to prevent neural transmission) and all the major blood vessels in the neck (to prevent blood supply to the brain). Even when these conditions are met, cervical dislocation raises concerns about animal welfare; visual evoked responses can be obtained from hens for up to 4 min after dislocation, depending on the method (stretching the neck causes loss of the response more quickly than crushing, allegedly due to concussion of the brain) (Gregory and Wotton, 1990). It is therefore preferable that if an animal is to be killed by decapitation or cervical dislocation, it should first be rendered unconscious.

B.2. Gaseous anaesthetic overdose

Exposure to carbon dioxide (CO_2) is a method of killing that has been widely recommended for small farm and laboratory animals and birds, however, there are aspects of its physical characteristics and the physiological responses induced by this gas that raise welfare concerns. CO_2 is an acidic gas that is known to cause irritation to mucus membranes, it has a pungent odour and can cause a profound sense of breathlessness before inducing unconsciousness in humans. Inhalation of this gas can cause animals to exhibit an excitation phase which might be caused by the animal experiencing a sensation analogous to suffocation since respiratory systems are stimulated by increasing CO_2 concentrations in the blood. However, there is some debate as to whether the animals might already have become unconscious at this point (Jongman et al., 2000). Pigs will voluntarily experience 72 h water deprivation or 24 h food deprivation rather than experience a second exposure to CO_2 (Raj and Gregory, 1995; see also Jongman et al., 2000). The degree of aversion to CO_2 varies with concentration (Raj and Gregory, 1995; see also Hewett et al., 1993; Hackbarth et al., 2000) and aversion has also been reported in several other species, e.g. aquatic mammals, birds and laboratory rodents (Raj, 1996; Cooper et al., 1998; Leach et al., 2001), but the degree of CO_2 aversion is both species- and concentration-dependent.

B.3. Hypoxia or anoxia

Various gases have been used to deplete oxygen and so cause hypoxia or anoxia. Evidence suggests that hypoxia or anoxia is a humane way of inducing unconsciousness and death, but problems can arise when the gas used to substitute the oxygen has aversive properties, or the species of animals to be euthanased has physiological compensatory mechanisms (resilience or tolerance to hypoxia).

Argon is odourless, tasteless and appears to cause no aversion in pigs. A mixture of 30% CO_2 in argon causes rapid loss of brain function in chickens (Raj et al., 1992) turkeys (Raj and Gregory, 1994) and pigs (Raj et al., 1997), although this is reportedly aversive to laboratory mice and rats (Leach et al., 2001).

Nitrogen has also been used to induce anoxia. Because this has a density very similar to air it must be used in a closed container. A mixture of mostly nitrogen (>80% by volume) and a small proportion of argon has been recently implemented for killing meat chickens under commercial conditions (Raj, personal communication).

Carbon monoxide (CO) causes rapid death through hypoxia and there is little apparent distress caused during the induction of unconsciousness in terrestrial species. CO must be supplied using a pure commercial source because other sources usually contain additional substances that might interfere with the effectiveness of CO hypoxia. CO is toxic to humans and therefore the concentration both inside and outside the chamber should be monitored.

Hypoxia (induced by CO, nitrogen or argon) can cause vocalisations and convulsions after loss of consciousness that may be aesthetically unpleasant. Due to physiological and metabolic differences, hypoxia may not be the best killing method for rodents, reptiles, aquatic animals and birds.

B.4. General

Close et al. (1996) and the AVMA Panel on Euthanasia (2000) list a wide variety of methods considered to be unacceptable for killing vertebrates. These include hypothermia (e.g. ice-slurries for fish and other heterotherms), hyperthermia, drowning or removal from water, neck crushing, nitrous oxide, strangulation, ether, chloroform, sedatives (due to the large volume required), oral agents (administered in drinking water) and certain narcotic agents.

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