



International Society for Applied Ethology

Ethical Treatment of Animals in Applied Animal Behaviour Research

Applied animal behaviour studies are of fundamental importance to developing our understanding of animals. The use of animals in such studies does, however, raise important ethical issues. Many applied behaviour studies are non-invasive and based on observing the animals in the environment that they would normally be found. But behaviour research may also require manipulation of the animals and/or their environment. To help ISAE members make what are sometimes difficult decisions about the procedures involved in their studies, ISAE has an Ethics Committee and ethical guidelines. The guidelines are to be used by researchers, conference organisers, scientific reviewers and the Ethics Committee in planning and reviewing research.

The members of the Ethics committee (2023):

Fernanda Tahamtani (Chair), Norway
Cecilie Mejdell, Norway
Elize VanVollenhoven, South Africa
Beth Ventura, UK
Ellen Williams, UK
Inès de Freslon, Chile
Bianca Vandresen, Canada

The Guidelines are printed below. Any comments can be e-mailed to Dr. Fernanda (Faye) Tahamtani (fmt@animalia.no).

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Guidelines for Ethical Treatment of Animals in Applied Animal Behaviour and Welfare Research.

1. Background

The International Society for Applied Ethology (ISAE) is a professional organisation of members with primary research interests in applied studies of animal behaviour and related disciplines. Because the work of its members requires the use of animals, the Society has had a continuing interest in promoting the welfare and ethical treatment of animals being used in research. This is particularly relevant as many ISAE members are involved directly in studying animal welfare and ethics, or are involved in relevant committee work or legislative procedures. ISAE members are therefore required to have greater insight in these issues than the average animal researcher, and may also be expected to develop their own research at high ethical standards. The ethics guidelines were initially developed to provide ISAE members with a basis for structured self-evaluation of the ethical nature of their work, and to serve as guidance when planning research involving the use of animals. They are also an important instrument for referees in assessing the ethical nature of abstracts submitted to ISAE congresses.

ISAE members 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.

2. Introduction

The use of animals in research and education has attracted ethical concern for many years, most notably in biomedical research and testing, but over time ethical concerns have been raised over less invasive studies such as animal behaviour research (Mench, 2000). This raises a strong need for justification of the use of animals in behavioural research, and some guarantee that the research is conducted in an ethically acceptable manner. 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 standards for work to be published, requirements for funding proposals to have statements that ethical guidelines will be adhered to, and the increasing public requirement for 'transparency' of research. Issues of concern include (but are not limited to) whether the purpose of the experiment justifies the use of animals, what the animals will experience, whether the expected level of suffering can be considered acceptable in that particular context and whether the experiment is designed so as to reliably answering the research questions.

3. Legislation

It is recognised that most ISAE research is carried out under national legislation regulating the use of animals in research. However, ISAE as an organization and many individual ISAE members are directly involved as experts in policy-making, and many ISAE members carry out research on which animal welfare legislation is based. Given this, the minimum standards set by legislation may not always be sufficient to meet the high standards expected of ISAE as professionals in a privileged position in the context of animal welfare policy. It is hoped these guidelines will therefore serve to promote and progress animal welfare and ethics, rather than just following 'rules' of legislation which may be minimum standards. To ensure

the highest welfare and ethical standards, investigators should remain apprised of current relevant literature and conduct their research according to the spirit and letter of their local legislation as well as the spirit of the ISAE Guidelines. Investigators should also consider whether other types of ethical approvals are needed for their research. This may include the need for approval from a human research ethics committee, for example where surveys are conducted or other types of personal data are presented which may need appropriate protection. Guidelines on, and necessity to seek this approval will vary by jurisdiction. It is good practice in reporting to quote relevant ethics approval numbers from these committees, or state that no approval was required by the relevant granting authority (e.g. ethical waiver). Ethical approval should be acquired before the start of data collection.

4. Ethical Stand-Point & Decision Models

By definition, ISAE members 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 at least some research purposes, for the benefit of humans, other animals and sometimes the research subjects themselves. In the wider animal ethics discussion, this is only one of several positions regarding what is ethically acceptable for humans to do to animals. Given the importance of animal ethics in the context of animal welfare research and policy-making, ISAE members are recommended to make themselves familiar with different ethical views on animals (see Further reading below).

Legislation regulating animal research is usually based on the approach that such research is acceptable if there is no alternative means to obtain the same knowledge, if the harm to the animals is minimized and if the expected benefits justify the means.

To determine whether the benefits of research outweigh the harm, a harm-benefit analysis can be performed. The 'harm' is 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 humans, other animals or the environment. However, the principle also implies that the ethically acceptable option is the one that provides most benefits and involves the least harm. Therefore, when planning a study, the aim should not be to simply reduce harm to a level lower than the benefits, rather, the harm should be minimized (see 'Reducing the Harms') and the benefits maximised as far as possible (see 'Increasing the Benefits'). We emphasise here that this harm: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.

In 2016, an international working group on harm-benefit analysis for research with animals presented a review of different models for assessing harm and benefit and weighing them (Brønstad et al 2016) and proposed a formal model (Laber et al 2016) for assessment and decision making. Applying decision models to your own research can be an enlightening exercise as it can help you to analyse the harm:benefit ratio of 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 they believe 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 Harm

A widely accepted method for reducing the harm associated with animal research is implementation of the

Three Rs, i.e., Replacement, Reduction and Refinement (Russell and Burch, 1959).

5.1 Replacement

Replacement means 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 mannequin animals, video-recordings, etc. can be used in some circumstances. A replacement of sorts can be obtained by using animals that already live under the circumstances the researchers want to study, e.g. 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 keeping the number of animals to the minimum necessary to achieve the aims of the research. This sounds simple, but Reduction is arguably the most challenging principle (for a discussion of this, see Olsson et al 2012). From the philosophical perspective, if the animals are not suffering from being part of the study, it is not obvious why using fewer animals is ethically preferable. And from the scientific perspective, animal numbers should not be reduced to so few that the results become statistically invalid – and unfortunately it is much more common to use fewer animals than statistically required than to use too many. There are several ways to reduce the number of animals without losing statistical validity.

5.2.1 Previous work:

If similar work has been conducted previously, this can sometimes 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 should be considered if it is to be used in this way.

5.2.2. Statistical methods:

The number of animals used can be reduced by good experimental design, appropriate observations and statistical procedures that enable several factors to be analysed with the smallest number of animals (Festing 2012). As a very minimum, statistical power analysis should be used to determine the smallest number of animals that need to be used in a study in cases where variance is known or can be predicted.

5.2.3 Epidemiological approach:

Sometimes it will be possible to study the spontaneous occurrence of behaviours as they occur on farms, zoos, in the wild, etc. This means no extra animals will be required for the purposes of research. Such studies can help identify which parameters are most likely involved in a particular behaviour, and can therefore help 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. Most legislation protecting animals covers vertebrate animals and a few invertebrate species such as cephalopods. The possibility that other invertebrates such as spiders might experience pain or an analogous sensation (reviewed by Sherwin, 2001) should also be considered.

5.3.1 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 it can be obtained using an alternative approach which does not impose suffering on animals specifically for the experiment in question (for example, can data be obtained from animals which undergo the procedure as part of animal husbandry or as part of a different experiment?). Pain, suffering or distress should be minimised both in duration and magnitude to the greatest possible extent, but without jeopardising the aims of the experiment. Some species are 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 to avoid showing evidence of pain or injury, presumably to avoid being targeted by predators.

In research involving surgery, pre- and postoperative care should 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 endangers the experimental aims. For example, studies that test novel anaesthesia/analgesia protocols should compare these to the current most effective standard of care as established by the literature, regardless of legal local practices. In addition, the inclusion of sham treatments (i.e. negative pain controls) should be considered instead of positive pain controls. In such cases where positive pain controls are essential to the aims of the study, all animals should receive comprehensive pain treatment as soon as the necessary data are collected. The use of neuromuscular blocking agents alone is generally unacceptable.

Drawing the line between sentient and non-sentient animals is a difficult challenge. The current scientific consensus seems to be that all vertebrate animals and some invertebrates (primarily cephalopods and decapods, see Broom 2013, but also increased research on insects, see Klobučar and Fisher 2023 and Gibbons et al. 2022) are capable of experiencing suffering of one kind or another. This will be influenced by many factors such as the species, age, sex, reproductive condition, social status, individual experience, perceptions, motivations and natural behaviour of the animal.

5.3.2. Housing:

Standard housing of animals in farms, zoos and laboratories is often minimalist and designed primarily for the convenience of humans. This can often result in the animals exhibiting behavioural or physiological responses indicative of reduced welfare, although it is difficult to assess how great this impact is. Housing animals under minimalist standard conditions is itself likely to cause a degree of suffering - even before any experimental procedures have been conducted. It might be argued therefore, that any study which requires housing animals under standard conditions causes suffering, even if the experimental procedure itself appears to cause none. 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. The environmental and physical

conditions (e.g. temperature, humidity, air quality, etc.) should also be considered. Animals kept in experimental facilities should be kept in their temperature/humidity comfort zones and/or have access to shelters (from e.g. sun/rain/wind) at all times.

Housing that prevents animals from performing important behaviours will not only have negative consequences on animal welfare but also lead to animals that are often less adequate research subjects because they are stressed and behave abnormally. Hence, adapting housing to the behavioural biology of the animals ("environmental enrichment" should always be a priority (e.g. Olsson et al 2003).

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. Again, we emphasise that welfare implications of housing, husbandry and other conditions not only during but also prior and subsequent to the experimental phase should be considered in the ethical justification of a study.

5.3.3 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 the choice may require balancing the impact of a more invasive long-lasting method and the need for 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, and the 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.

5.3.4 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.

5.3.5. Presence of experimenters and handling:

The presence of humans can have a considerable effect on the behaviour of animals, which needs to be taken into account in terms of animal health and stress (e.g. disease transmission, disturbance) as well as validity of the behaviour data. The effect may be positive or negative, depending on previous experience of the animals and the nature of the human-animal relationship in question. 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 responses and learned aversion to

handling in the future; potentially, this can invalidate the research. Investigators should familiarise themselves with the appropriate handling methods for the animals to be used.

5.3.6 Final disposal and euthanasia:

In applied ethology research – unlike much biomedical research – many studies do not require that animals are killed in the end. 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 should 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 hygiene consequences. Similarly, some species might be placed into private homes or sanctuaries.

It may be considered to place field-caught animals in zoos or reserves to allow research without 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 hygiene 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 them or to existing populations.

It is however not clear that reducing animal numbers by re-using animals is the ethically preferred approach – and this points to a dilemma between refinement and reduction. Re-use leads to an increase in accumulated interventions in the individual animal, and if these interventions cause pain or distress, the overall impact on an individual animal is just as important as the total number of animals. In the case of animals caught in the wild, not only procedures on them but also the effect of captivity housing must be included when the accumulated effect is estimated.

Whether animals are killed, and the manner in which they are killed are significant components of the ethical acceptability of a research programme. Euthanasia (humane killing without pain or stress) should of course be practised where possible. In applied ethology studies, the method of the animals' death may not be fully under the control of the investigator, e.g., animals on farms will usually remain on the farm and will be slaughtered commercially. This does not mean that the investigator has no responsibility for how these animals are treated. If the investigator has control over the method of killing, 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. 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.

5.3.8 Procedures:

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. Investigators should consider choosing welfare-based end-points that allow effective prevention of more serious animal welfare problems, 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. Death is considered to be an unacceptable end-point.

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 in order to be able to interrupt if unacceptable levels of suffering occur, in which case 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 become totally unresponsive although aware and cognisant of their surroundings (tonic immobility). Field studies should be considered as an alternative method of investigation.

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, comfort behaviours, 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. In general, to avoid chronic hunger, it is preferable to deprive an animal of food for a pre-determined period of time before testing, rather than attempting to achieve and maintain an arbitrarily specified target bodyweight.

Adverse Conditions: Studies aimed at inducing adverse conditions in animals are sometimes conducted to gain knowledge of applied problems, e.g., parasite loads, pesticides or homeostatic challenges. These procedures may cause suffering and again 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.

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 'Housing' 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 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, research methodology, equipment, housing and trained personnel

are all of an adequate standard and available for the duration of the study.

6.2 Significance of the aims

The aims can be of various forms, for instance, health or welfare of humans or non-human animals, economic gains for livestock, 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 study on feather-pecking might be able to state 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. It may be more difficult to state the likely benefits resulting from fundamental research. However, although it may be hard 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 Reviewing 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. For guidance on how to approach this systematically, see for example Hooijmans (2014).

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. The way the results are reported in a paper is also of ethical importance. Concern over insufficient quality of reporting has led to the development of guidelines, with the ARRIVE guidelines being the most well-known and widely adopted. To increase transparency and promote application of refinements, it is also important to describe interventions with animals in sufficient detail and to describe measures taken to reduce the negative effect on animal welfare. 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.

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. Researchers exposing animals in field studies to particular stimuli (e.g. sounds, lights, human disturbance, etc) should assume that other species in the area will also be exposed and ethically consider this during the design of their study. The welfare of other animals dependent on the subject (e.g. offspring) should also be considered. Guidance on conducting field experiments is available in Gannon & Sikes (2007) and Home Office (2016).

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9. Further reading

9.1 Ethics/Codes of practice/Guidelines

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Webster, M.M. and Rutz, C. 2020. How STRANGE are your study animals? *Nature* 582: 337-340. <https://doi.org/10.1038/d41586-020-01751-5> Supplementary information: <https://media.nature.com/original/magazine-assets/d41586-020-01751-5/18082298>

9.2 Animal Numbers

Festing, Michael.F.W. 2016. *The Design of Animal Experiments. Reducing the use of animals in research through better experimental design*. 2nd edition SAGE Publishing.

National Centre for the 3Rs Experimental Design Assistant – EDA. <http://www.nc3rs.org.uk/experimental-design-assistant-eda>

An interactive short course on experimental design for research scientists working with laboratory animals <http://www.3rs-reduction.co.uk/>

9.3 Pain, Suffering and Distress

Sneddon, Lynne U., Robert W. Elwood, Shelley A. Adamo, and Matthew C. Leach. 2014. *Defining and*

assessing animal pain." *Animal Behaviour* 97: 201-212.

Guidelines for the Recognition and Assessment of Animal Pain. <http://www.vet.ed.ac.uk/animalpain/>

9.4. Killing methods

AVMA Guidelines for the Euthanasia of Animals: 2013 Edition
<https://www.avma.org/kb/policies/documents/euthanasia.pdf>

9.5 General Animal Welfare and 3Rs

Appleby Michael C, I. Anna S. Olsson and Francisco Galindo. 2018 *Animal Welfare*. 3rd edition. CABI Publishing. Planned publication date Summer 2018.

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