Resources for AWERB members

RSPCA Animals in Science Department June 2023

Humane killing

Aim of this resource

To help AWERBs discuss local practice for humane killing, in general and when reviewing project applications.

Relevant AWERB tasks

Advising staff on animal welfare and the application of the 3Rs, supporting named persons and others dealing with animals, advising on rehoming, promoting a Culture of Care.

Recommendation

Regularly review and discuss humane killing techniques, equipment, and training, as well as issues around humane killing.



The issue

The end of an animal's life is part of their overall lifetime experience and should be considered for each project application. Permissible methods of killing are set out in Schedule 1 of the ASPA, but these should still be reviewed by the AWERB – both within each project and when deciding what is acceptable within the local Culture of Care.

Note: the term 'humane killing' is used instead of 'euthanasia'. This is because the latter usually refers to killing to end suffering and for the benefit of the individual, which is not always the case for animals in research and testing. Key points - general:

- It is good practice to periodically review methods and protocols for humane killing at the establishment, including techniques, equipment, and training.
- The aim should be to cause no more than threshold suffering preferably less.
- Killing animals is a *process* with multiple steps to consider.
- Practical factors, such as speed and economics, may need to be considered but animal welfare must always take precedence.
- Killing animals can involve significant 'emotional labour' for staff. It is essential to consider the views and feelings of staff members and provide appropriate support.

Key points for project review:

- When reviewing applications, the first question should always be: do the animals have to be killed, or are there alternative options?
- The AWERB should always discuss humane killing techniques, even if an application cites Schedule 1 methods.
- The most appropriate method will depend on multiple factors, including whether there is a scientific requirement for using a particular technique.
- If the killing method has been competently and effectively applied, the method for completing killing and confirming death should make no difference to the animal.

Background information:

- It is good practice to periodically review standard protocols for humane killing at the establishment, including techniques, equipment, and training. The NACWO, NIO, and NVS¹ should be aware of current literature on the welfare implications of different techniques, and refinements, and should bring these to the AWERB. You could suggest a review as an annual agenda item. The experience of the animal should be the primary consideration, but it is also important to discuss how staff feel about each technique. The views of UK laboratory animal personnel on rodent killing methods were surveyed in 2023 and various issues were highlighted, including lack of availability of certain methods [1]. You could suggest a review of which methods are available at your institution.
- The aim should be to cause no more than threshold suffering preferably less. The threshold for regulation under ASPA is 'a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice'. Minimising suffering does not always mean inducing unconsciousness as rapidly as possible. It is the animal's total experience, from initial capture to final loss of consciousness, that counts.
- Killing animals is a process with multiple steps to consider. Is there a need for capture, handling, and restraint? Must the animal be removed from the home cage or tank? Will they be mixed with unfamiliar animals? Is application of the method painful or distressing, even mildly? What does the animal experience between application of the technique and loss of consciousness? How will death be confirmed, and how soon? What is the success rate of the method? The welfare impact of each of these steps can be used as a focus for refinement and to help decide which method to use.
- Practical factors, such as speed and economics, may need to be considered but animal welfare must always take precedence. Using faster techniques for killing is acceptable only if no additional suffering is caused to animals. If choosing between techniques that have different costs, you could ask to consider whether the money saved could be spent on improving welfare or staff training.
- Killing animals can involve significant 'emotional labour' for staff. It is essential to consider the views and feelings of staff members and provide appropriate support. People may be upset by killing certain species, having to use physical methods, or being asked to kill surplus animals, especially if that surplus could have been avoided. The AWERB should discuss these issues and ensure that all staff are informed of both the scientific benefits of projects and the justification for given techniques. You could ask how surplus animals are avoided, for animal and human welfare and ethical reasons. Providing staff with appropriate support is an AWERB task and part of a good 'Culture of Care' [2]; see also the 'Cost of Caring' resource [3].

¹Named Animal Care and Welfare Officer, Named Information Officer, Named Veterinary Surgeon.

Project review:

- The first question should always be: do the animals have to be killed, or are there alternative options? It may be possible to rehome or release animals [4], or under certain circumstances it may be ethical to reuse them. These alternatives have to be written into the application. The AWERB should advise on rehoming schemes (p. 39-4 in [4]). If there is no alternative to humane killing, you can ask whether tissues will be shared, to avoid killing animals for this purpose.
- The AWERB should always discuss humane killing techniques, even if an application cites Schedule 1 methods. All techniques cause a degree of anxiety and discomfort, and the applicant should be able to justify the method(s) and explain how distress will be minimised.
- The most appropriate method will depend on multiple factors, including whether there is a scientific requirement for using a particular technique. Other relevant factors include the animal's experience, the species, stage of development, and size of the animal, the training and competence of staff, and the availability of equipment. For example, if brain tissue is required for scientific reasons then physical methods may not be suitable. You may find this decision flowchart helpful (click here).
- If the killing method has been competently and effectively applied, the method for completing killing and confirming death should make no difference to the animal. However, if the animal has not been effectively killed, waiting for the onset of *rigor mortis* to confirm death may cause suffering if the animal is still conscious.

Schedule 1 methods:

There are three broad categories of methods for the killing of non-fetal animals in Schedule 1 [5]:

- Anaesthetic overdose: anaesthesia can be given by injection or inhalation, and is generally considered to be humane. However, some anaesthetics are aversive or irritant [6], so you could ask whether this has been checked for the species and strain. For example, intraperitoneal injection of sodium pentobarbital can be painful and irritating unless a chemical 'buffer' is added. The anaesthetic MS222 is also an irritant to zebrafish [7]. If a method involves catching, transporting, or restraining animals, you could ask whether they could be killed in their home cage or whether prior sedation might be appropriate.
- Carbon dioxide exposure: carbon dioxide was thought to be both humane and 'aesthetic', but there is now evidence that it causes pain and distress [7]. It was previously thought that the 'least worst' protocol was to introduce 100% CO₂ at a flow rate of 20% of the chamber volume per minute [8,9]. However, new American Veterinary Medical Association guidelines suggest a rate of 30-70% chamber volume per minute [6]. The aim is to ensure that animals are unconscious before the CO₂ causes a burning sensation, but they may still experience distressing dyspnoea ('air hunger') and anxiety [6,11,12]. Potential refinements include delivering carbon dioxide to home cages where possible (or placing home cages in the chamber), keeping animals in established groups, researching the benefits of anaesthetising animals first, using a diffuser, and warming the carbon dioxide for conscious animals.
- Physical methods cervical dislocation, concussion of the brain: good technique is absolutely crucial for these methods. They can cause the least suffering of all if done properly or extreme suffering if ineffectively applied. Operators should be trained, competent, and willing to use physical methods. Success rates (whether animals stop breathing almost instantly) should be monitored. The establishment should have a set acceptable success rate and operators who cannot maintain this should be retrained or not permitted to use the technique.

Gradual decompression as a new technique for humane killing of rodents

Gradual decompression refers to a slow decrease in atmospheric pressure, similar to the experience of being at high altitude. This method has been used to stun chickens in agricultural settings and is considered a high-welfare alternative. A study compared gradual decompression with exposure to carbon dioxide in mice [13]. Gradual decompression was associated with fewer signs of pain and/or anxiety before loss of consciousness than carbon dioxide. Although this is not currently a Schedule 1 killing method, decompression may be a viable and effective refinement technique and is an emerging area of research to watch out for.