THE SWEDISH 3Rs CENTER

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Support in choosing method of euthanasia for mice

Photo: Sören Andersson/2See AB

Contents

Background

This support material has been compiled by the Swedish 3Rs Center on behalf of the Swedish National Committee for the Protection of Animals used for Scientific Purposes. The material is intended for anyone working with mice as research animals. The material is based on scientific literature on approved methods of euthanasia for mice used in research and has been compiled by the Swedish 3Rs Center together with the National Committee's group of experts. The Swedish 3Rs Center has also been in contact with Swedish research animal facilities where research using mice is conducted to collect information regarding their experiences of pre-euthanasia anaesthesia, euthanasia and control methods. The experiences collected from research animal facilities are listed for every method of euthanasia within this material. The experiences are not reproduced as citations, but here formulated according to the structure of this material.

This material includes information regarding methods, approved in Swedish research animal legislation, of anaesthesia, euthanasia, and confirmation of death in mice. Methods of anaesthesia are described in the section "Anaesthetics" followed by sections describing the approved methods of euthanasia. Methods to confirm death in mice are collected in the section "Control methods".

All sections contain recommendations based on scientific literature and most sections also contain practical insights collected from Swedish animal facilities focusing on mice as research subjects. Reference literature can be found at the end of this document and can be used for further reading regarding the described methods.

The support material was originally published in December 2023 and has been updated in March 2025.

Introduction

Euthanasia

Euthanasia of mice refers to any intentionally induced process that results in the death of a mouse. Most mice bred for animal experimentation will be euthanised for various reasons. Euthanasia of an animal can be executed, for example, due to selection of genetic variants, breeding excess, or the fact that an experimental endpoint has been reached. Euthanasia can also be a prerequisite for collection of tissues and organs at termination of animal studies.

Choice of method

Euthanasia is a process that includes several events that can be perceived as stressful or painful for the mice. Examples are capturing, handling, fixation, separation of mice housed in groups, housing of mice together in new groups, new and unfamiliar surroundings, transportation, and the execution of euthanasia *per se*. Previous experiences from experimental procedures are of importance as research indicates, for example, that mice that have undergone inhalation anaesthesia become pronouncedly more stressed if subjected to the procedure repeatedly. It can be difficult to completely avoid stress during euthanasia, but it is of utmost importance to minimize the stress for the animals.

When choosing method for euthanasia, there are several different aspects to consider, such as

• the purpose of the experiment

• the age, sex, strain and any injuries or diseases that the animals may have

• the location where the euthanasia will take place and the equipment you will have or could get access to there

• what methods the staff who will carry out the euthanasia are trained to perform.[1]

When you have considered the above factors, you may find that one or more approved methods of euthanasia are more suitable in your particular situation. Of the methods that are suitable, you should choose the one that has the least negative impact on animal welfare.

In case of an emergency, where a mouse must be instantly euthanised, other methods than those described in this document can be applied.[2]

[1] 12 ch. 6 § SJVFS 2019:9.

[2] 12 ch. 2 § SJVFS 2019:9.

Recommendations for reduction of stress associated with euthanasia of mice

- Handle mice with care and avoid handling the mice by the tail or unnecessary capturing. Carefully handled mice will not be as distressed.
- If inhalation is used for anaesthesia or euthanasia, use the home cage as the inhalation chamber if possible.
- Avoid grouping of mice that have not been kept together before euthanasia.
- Avoid euthanasia in close proximity to other mice, as those animals can be affected negatively and display signs of distress.
- The person performing the euthanasia must be educated, trained and comfortable using the method of choice.
- If possible, dim the lighting when handling awake mice.

About the person executing the euthanasia

The person performing the euthanasia must be educated and trained in the method used[3] to be able to execute the euthanasia with the least possible negative impact on animal welfare. It is also of importance that the person who performs the euthanasia feels comfortable with the method and the actual euthanasia, for the welfare of both the person and the animals. The person performing euthanasia must be trained in recognising signs of pain and distress and be able to confirm unconsciousness and death in mice.

People euthanising animals can be affected both emotionally and psychologically. Physical methods of euthanasia can have a more pronounced impact of a person performing euthanasia compared to a non-physical method. A phenomenon that can occur is compassion fatigue. Compassion fatigue may emerge as a result of prolonged exposure to the emotional strain of caring for animals, particularly when they undergo experiences of fear, pain, or anxiety, such as during experimental procedures or euthanasia. A person affected by compassion fatigue can become depressed, frustrated and have a limited ability to provide animals the care they need, which in turn can affect animal welfare in a negative manner.

Established routines and long-term planning are important to counteract compassion fatigue. Below is a list inspired by proposals from the North American 3Rs Collaboratives aimed at mitigating compassion fatigue:

- Encourage and respect each other’s boundaries between work and personal life. Help each other and arrange activities to increase motivation and collaboration.
- Respect and value the bond that can occur between laboratory animals and staff working with them. Appreciate the animals' value and contribution to research progress, consider arranging an animal memorial site.

[3] 12 kap. 6 § SJVFS 2019:9.

- Encourage and schedule positive interactions with the laboratory animals such as training and play.
- When possible, consider rehoming of animals as an alternative to euthanasia.
- Promote self-care such as good nutrition, recovery, sleep and exercise.
- Help and support each other by taking turns with mentally stressful tasks such as euthanasia.
- Work according to the 3Rs principle, finding new ways to refine, replace and reduce animal experimentation.
- Advocate an open dialogue about animal testing, both at work and in communication with the public.
- Promote an open communication where everyone feels safe to share their thoughts and opinions.

Methods of pre-euthanasia anaesthesia

Anaesthetic agents are used to induce anaesthesia prior to euthanasia of mice or directly as a method of euthanasia in itself. Information regarding use of anaesthetic agents as a method for euthanasia can be found in the section ‘Anaesthetics’.

Methods of euthanasia of mice

The following methods are approved for euthanasia of mice:

- Exsanguination of unconscious animals
- Decapitation
- Cervical dislocation
- Inert gases
- Injection of chemical agents into unconscious animals
- Carbon dioxide
- Fatal blow to the head
- Overdose of anaesthetics

Some of the methods require special circumstances or are associated with specific restrictions.[4]

Control methods

Following euthanasia, it is crucial to confirm the animal's death before proceeding with any subsequent actions such as sampling or disposal of the body. Approved control methods for mice are confirmation of permanent cessation of circulation or

[4] Table 1, 12 ch. 8 § SJVFS 2019:9.

the onset of rigor mortis, provided there is no risk of suffering. In cases where neither cessation of circulation nor rigor mortis can be used, death can be ensured by destruction of the brain, exsanguination, or cervical dislocation.[5]

Experiences from Swedish animal facilities

Collected here are experiences that apply to several different methods of euthanasia. Experiences that are specific to a certain method are described under the relevant sections for the methods.

- We have designed procedures that are easy to follow and minimize stress throughout the euthanasia process.
- When we transport mice to the room for euthanasia, we do so quietly, with dimmed lighting or by covering the top of the cage to protect the mice from bright light.
- If possible, I prefer to euthanize mice in the anteroom outside the animal room so that other mice do not hear sounds or smell odours from those being euthanized.
- For mice that need to wait before euthanasia, I cover the cages with a towel, surgical drape or similar. This reduces the visual impact and makes it darker, which can be calming for the mice.
- We talk to students and new PhD students about euthanasia before they start working with the animals. We also do a follow up after a while on how it feels for the students.
- We plan euthanasia of animals so that there is plenty of time to carry it out without stress. We don't want to risk negatively affecting the animals with our stress.
- When many animals are euthanized during the same week, we recommend our staff to divide the euthanasia between several days. This is only done in cases where the animals are not at risk of suffering. When possible, the work with euthanasia can also be divided between other tasks. It can be mentally stressful to perform euthanasia of animals for a long period of time without breaks.
- To reduce the stress of the animals, we think it is important that they are euthanized by a calm person in a calm environment.
- We make sure that all equipment, medicines, etc., are well-prepared before the euthanasia begins.
- I practice a lot to feel confident in the method I use.

[5] 12 ch. 7 § SJVFS 2019:9.

Exsanguination of unconscious animals

General information

- The method is approved for mice at all ages.[6]
- The mouse must be unconscious before euthanasia is initiated.[7]

The method in brief

Exsanguination procedures should exclusively be conducted on unconscious mice and entails opening the carotid arteries (an uncommon practice in mice) or at least one of the central blood vessels[8], such as the main body artery, using instruments like scissors or a scalpel.

Animal welfare

Since the mouse must be unconscious during exsanguination, it is primarily the choice of anaesthetic agent and depth of anaesthesia that is decisive for animal welfare during exsanguination. The mouse may also be unconscious due to other reasons, such as injury or illness. To ensure that the mouse is unconscious, the pain response can be tested. One way to assess the pain response is to test the avoidance reflex by firmly pinching a paw. If the mouse is unconscious, it completely lacks the reflex to pull its leg. The avoidance reflex should be tested shortly before bleeding begins as the depth of anaesthesia may vary over time. The test should be done in the hind paws as research shows that the reflexes of the hind legs are better indicators than those of the front legs. It is important that unconsciousness is ensured before exsanguination as exsanguination leads to circulatory failure, which is an anxiety-inducing condition.

Physiology

During exsanguination, the blood volume decreases drastically and the blood pressure drops. It causes circulatory and organ failure, eventually leading to death.

Practical aspects

The equipment should be prepared for anesthetizing the animal prior to exsanguination. To open the blood vessels, tools such as scissors or a scalpel are required. It is essential to regularly inspect and maintain the equipment, ensuring it remains sharp to facilitate swift and effective euthanasia procedures.

[6] Table 1, 12 ch. 8 § SJVFS 2019:9.

[7] Table 1, 12 ch. 8 § SJVFS 2019:9.

[8] 12 ch. 26 § SJVFS 2019:9.

Recommendations

Selection of an anaesthetic agent and careful monitoring of the animals during anaesthesia are paramount, constituting the cornerstone in ensuring animal welfare within this euthanasia method. It is also important to recognize that physical euthanasia techniques like exsanguination may induce a more pronounced negative psychological impact on the individual performing the procedure, in contrast to non-physical methods. It is essential that the individual conducting euthanasia feels comfortable with the method and is willing to perform it, ensuring both their own mental well-being and the welfare of the mice.

Experiences from Swedish animal facilities

- We think it is important that the mouse remains in the anaesthetic mask during the entire procedure. Only when the mouse is bled and has died the mask will be removed.
- Within our organization, this method is part of a practical training that everyone needs to pass with a passing result before they get the opportunity to work single-handed with the animals.
- Our "standard euthanasia method" for animal technicians includes clipping of the diaphragm and heart to ensure death, and we do this on all animals we euthanize. The method is good in that it is safe, but quite time-consuming when euthanizing many animals in a day.

Decapitation

General information

- Decapitation may be a suitable method of euthanasia for mice that are 10 days old or younger, as few other methods are possible to use for mice that young.
- The method is an approved method of euthanasia for mice of all ages when other methods are not possible to use.[9]
- The method necessitates handling the mice.

The method in brief

Decapitation means that the head is quickly separated from the body with the help of a guillotine, scissors or the like.

Animal welfare

Decapitation is a method that quickly leads to unconsciousness and death when performed correctly. Research has so far been unable to show whether mice are conscious and experience pain or not after decapitation. There is a risk of unsuccessful decapitation which can be associated with suffering. To maintain good animal welfare, it is crucial that the method is carried out with the proper technique. When decapitating awake mice, they must first be captured and fixated, which may be associated with stress. Avoid performing decapitation in the vicinity of other mice as they risk being negatively affected and stressed.

Physiology

Shortly after decapitation, all brain activity ceases. The method triggers a strong sympathetic activity and release of stress hormones (catecholamines) from the adrenal medulla. The massive blood loss that decapitation causes results in a quickly induced lack of oxygen in the brain, leading to a state where no brain activity can continue. Decapitation causes no chemical contamination of tissues.

Practical aspects

Guillotines designed for mice are available for purchase. For mice 10 days old or younger, sharp scissors can be used. The use of a guillotine or other sharp tools poses a risk of injury to the person performing the euthanasia. Well-designed routines can reduce the risk of injury. The equipment must be regularly inspected and kept sharp to ensure a quick and efficient kill. An aid for fixation can facilitate the placement of the animal when euthanizing awake mice, as well as reduce the risk of injury to the person who perform the euthanasia. For instance, such

[9] Table 1, 12 ch. 8 § SJVFS 2019:9.

assistance could come in the form of a plastic cone in which the mouse is positioned.

Recommendations

Knowledge and skill in using the method is of utmost importance. To ensure that a good technique is maintained, regular training and follow-up is required. Decapitation training can preferably be performed on unconscious or dead mice, but it is important to remember that this is different from performing the method on animals that are awake. Physical euthanasia methods such as decapitation can have a greater negative psychological impact on the person performing euthanasia compared to methods that are not physical. It is essential that the individual conducting euthanasia feels comfortable with the method and is willing to perform it, ensuring both their own mental well-being and the welfare of the mice. When euthanising mice using this method, handling and fixation can induce significant stress. Stress levels can be minimized by avoiding lifting the animal by the tail during capture, lifting, or transfer and by handling the animal as little as possible and with care. Decapitation may be suitable for mice aged 10 days or younger, as few alternative euthanasia methods are practical at this age.

Experiences from Swedish animal facilities

- I use decapitation as a killing method for baby mice less than a week old. I am careful to euthanize the cubs at some distance from the parents to avoid that they hear the sounds that the cubs make. If euthanasia is not urgent, I move them to another room so that other mice are unaware of the euthanasia.
- When decapitating baby mice, I am careful to use sharp and sufficiently large scissors. I perform the decapitation firmly and without hesitation. To avoid accidentally cutting off the mouse's paws, I hold them firmly or bring the scissors from the neck forward towards the head, instead of the other way around.

Cervical dislocation

General information

- The method is approved for mice at all ages.[10]
- The method necessitates handling the mice.
- The method requires a lot of training and a good knowledge of the technique.
- The method is time-efficient when euthanizing single animals.

The method in brief

Cervical dislocation is a manual method of euthanasia that involves separating the neck vertebrae resulting in a fatal spinal cord injury.

Animal welfare

Cervical dislocation is a method quickly resulting in unconsciousness and death when performed correctly. To perform cervical dislocation on mice that are awake, they must first be captured and fixed, which may be associated with stress. To maintain good animal welfare, it is crucial that the method is carried out with the proper technique. There is a risk of failed cervical dislocation, which can cause suffering. Cervical dislocation performed by jerking the tail, without applying sufficient pressure to the neck, carries an increased risk of fracturing the spinal cord in the wrong position. A dislocation further down the spine can cause the mouse to continue to breathe, remain conscious and suffer. Avoid performing cervical dislocation in the vicinity of other mice as they risk being negatively affected and stressed. Research has not been able to show whether mice are conscious or feel pain after cervical dislocation, but this is considered unlikely given the damage that occurs to the spinal cord. If an incorrect dislocation is performed on unconscious mice, there is a risk that they will wake up to pain and suffering.

Physiology

When the neck vertebrae are separated, a fatal injury occurs in the spinal cord, breathing stops and brain death occurs. The technique causes no chemical contamination of tissues.

Practical aspects

There are different techniques for performing cervical dislocation, but the basic principle is the same. Cervical dislocation is a manual method which means that the mouse is first fixated by a firm grip at the root of the tail and with firm pressure on the neck, just behind the skull. When the mouse is fixed, the neck is dislocated with a definite movement and a downward and forward pressure on the neck, while

[10] 12 ch. 17 § SJVFS 2019:9.

the spine is stretched with a controlled pull on the root of the tail. Instead of a hand behind the skull, an aid, such as closed scissors or the like, can be used to fixate the mouse and carry out the euthanasia. In addition to any aids, no special equipment is required and the method can thus be performed at a low cost.

Recommendations

Knowledge and skill in using the method is of utmost importance. To maintain good animal welfare, it is crucial that the method is carried out with the proper technique. Practice on unconscious or euthanized mice is necessary, but it is important to remember that this is not the same as performing the method on animals that are awake. Physical euthanasia methods such as cervical dislocation can have a greater negative psychological impact on the person performing the euthanasia compared to methods that are not physical. It is essential that the individual conducting euthanasia feels comfortable with the method and is willing to perform it, ensuring both their own mental well-being and the welfare of the mice. When euthanised by this method, handling and fixation can be strong stressors for the mouse. Stress can be minimised by handling mice with care and avoiding the use of tail lifting during capture, lifting, and transfer.

Experiences from Swedish animal facilities

- Everyone in our research group must first practice on anesthetized animals to become confident in the method. If someone in the group does not feel comfortable with the method, an alternative method can often be used.
- I like to sedate the animal before euthanasia by cervical dislocation. If it is an emergency situation, I perform cervical dislocation without sedation to spare the mouse the stress of being sedated. I think it is important to be sure of the method and to carry out the euthanasia quickly. I like to use an aid that is narrow and hard, like a metal cage card holder.
- I only use cervical dislocation if the mouse is acutely ill to avoid transporting it to an anaesthesia station. If the mouse is sick and has a reduced general condition, I feel that the method is quick and simple. However, I am not comfortable performing cervical dislocation on awake, full-grown, healthy mice.
- I think it is important to handle the mice calmly and to practice the method a lot so that the dislocation can be performed quickly and without hesitation.
- If the grid on the cage is used as a base in the event of cervical dislocation, it is important that the grid is placed properly on the cage so that the surface is stable. We avoid euthanasia when there are other mice in the cage, we use an empty cage instead.
- When euthanizing by cervical dislocation, we always make sure that the cage mates cannot see or hear what is happening.

- I always let the mouse sit on something it can cling to like a grid or a ladder so that it sits firmly and doesn't try to run around.
- I am careful to keep a firm and good grip on the mouse to minimize the risk of it escaping and thus becoming more stressed. If the mouse seems anxious, I usually handle it with extra care. I do this by picking it up using a ladder or a tunnel and then holding it in my hand for a while before euthanasia.

Inert gases

There are several inert gases that can be used in euthanasia. Argon and nitrogen gas are commonly used.

General information

- The method is approved for mice at all ages.[11]
- The method is not suitable for mice that are 10 days old or younger.
- The method does not require fixation.
- Euthanasia can be performed in the home cage.
- Several animals can be euthanized at the same time.
- It is well known that inhalation of argon and nitrogen gas causes shortness of breath.
- From 4 December 2026, the method may not be used within the EU.

The method in brief

Inert gases displace the oxygen in the air. Inhalation of inert gases in high concentration causes oxygen deficiency and eventually death.

Animal welfare

Only a few studies have investigated how mice respond to euthanasia with inert gases. It is known that the lack of oxygen that occurs before the mice become unconscious causes respiratory distress. The discomfort it entails is likely to be amplified if the euthanasia also involves handling, transportation, unfamiliar surroundings or regrouping of the animals. These factors should be avoided as far as possible. Unlike carbon dioxide, nitrogen gas does not cause increased motor activity or trigger mice to exhibit jumping behaviour, which is indicative of significant stress.

Physiology

It has not been investigated at what concentration of inert gases oxygen deprivation occurs in mice. In rats, rabbits and mink, oxygen deficiency and eventually death occurs at a concentration >98% of argon or nitrogen. The lack of oxygen leads to respiratory depression and permanent brain damage. Alveolar bleeding has also been detected in rats, but whether this can occur also in mice is not known.

Practical aspects

Argon and nitrogen gas do not react with other substances and are not flammable or explosive. They do not have any smell or colour, making it difficult to detect

[11] Table 1, 12 ch. 8 § SJVFS 2019:9.

any leaks. Argon is currently a more expensive alternative compared to nitrogen gas. The gases are delivered in gas tubes with high pressure. Through a pressure regulator, gas lines and a flow meter, the gas can be led into a chamber where the mice are placed. The size, density, design and interior of the chamber, and the number of mice in the chamber, may affect how the chamber is filled with gas. This, in turn, can affect how quickly the mice become unconscious. The chamber should be designed in such a way that the mice cannot injure themselves. While euthanasia is in progress, the mice must be visually monitored.

Recommendations

Whenever possible, a high gas pressure into the chamber should be avoided as it can cause cold drafts and loud noises that can stress the mice. Also, to avoid mixing of mice from different cages and use the home cage whenever possible will contribute to reduction of stress. In cases of injury, illness, or stress, the respiration of mice may be compromised. When mice exhibit impaired breathing, it is crucial to administer appropriate anaesthesia prior to euthanasia with inert gas or consider alternative humane euthanasia methods. Failure to do so may subject the mice to heightened discomfort during the euthanasia process. Mice that are 10 days old or younger can continue to live for a long period of time despite lack of oxygen. Consequently, when euthanizing mice within this age range, alternative euthanasia methods should be used to ensure a humane and effective process.

Experiences from Swedish animal facilities

The Swedish 3Rs Center has not received any experiences from Swedish animal facilities regarding euthanasia using argon or nitrogen gas.

Injection of chemical agents into unconscious animals

General information

- The method is approved for mice at all ages.[12]
- The mouse must be unconscious before euthanasia is initiated.[13]

The method in brief

The mouse is injected with a chemical preparation intended for euthanasia of mice. During the injection, the mouse must be unconscious.[14]

Animal welfare

Since the mouse must be unconscious during the injection of a chemical agent, it is primarily the choice of anaesthetic agent and depth of anaesthesia that is decisive for animal welfare during euthanasia. The mouse may be unconscious due to other reasons, such as injury or illness. To verify unconsciousness in the mouse, the pain response can be evaluated. One method involves assessing the avoidance reflex by gently pinching a paw. If the mouse is indeed unconscious, it will exhibit no reflexive movement to withdraw its leg. It's essential to conduct this test immediately before administering the injection, as the depth of anaesthesia may fluctuate over time. The test should be done in the hind paws as research has shown that the reflexes of the hind legs offer more reliable indicators of unconsciousness compared to the front legs.

Physiology

The physiological effects vary greatly depending on the chemical agent injected. The preparation can, for example, have a paralytic effect or affect circulation and breathing.

Practical effects

Unconscious mice may be euthanized by injection of chemical agents that have no sedative effect and are specifically intended for mice and for this purpose. In exceptional cases, a regional animal research ethics committee can approve euthanasia using other chemical agents if there are scientific justifications. Some chemical agents may pose a risk to humans handling the chemical agents, such as neuromuscular blocking agents. Euthanised mice may need to be handled with care

[12] Table 1, 12 ch. 8 § SJVFS 2019:9.

[13] 12 ch. 12–13 §§ SJVFS 2019:9.

[14] 12 ch. 12–13 §§ SJVFS 2019:9.

depending on the chemical agent used to avoid risks to humans, animals or the environment.

Recommendations

The choice of anaesthetic agent and monitoring during anaesthesia is important as these are the most critical factors for animal welfare in this method of euthanasia. Read more about anaesthetic agents in the section ‘Anaesthetics’.

Experiences from Swedish animal facilities

The Swedish 3Rs Center has not received any comments from Swedish research animal facilities regarding euthanasia using chemical agents.

Carbon dioxide

General information

- The method is only approved for mice older than 10 days.[15]
- The method does not require fixation.
- Euthanasia can be performed in the home cage.
- Several animals can be euthanized at the same time.
- It is well known that inhalation of carbon dioxide causes shortness of breath and discomfort.

The method in brief

Inhalation of carbon dioxide in a concentration of 30% or higher causes unconsciousness and lack of oxygen which eventually leads to death.

Animal welfare

In the scientific literature, there is no clear answer as to whether a slow or rapid supply of carbon dioxide to the chamber is the best option. The discomfort is likely to increase if the euthanasia also involves handling, transportation, unfamiliar surroundings or regrouping of the animals. These factors should therefore be avoided as far as possible. When euthanized with carbon dioxide, unconscious mice can be mistaken for dead. It's imperative to confirm death, as unconscious mice may recover and regain consciousness, potentially leading to significant suffering. Especially if the mice wake up after being disposed or placed in a refrigerator or freezer.

Physiology

Euthanasia using carbon dioxide affects the body in several different ways, some of which are mentioned here. High carbon dioxide content in the inhaled air leads to an increased concentration of carbon dioxide in the blood, which in turn disturbs the acid-base balance in the body. The carbon dioxide lowers the pH in the blood, which primarily affects breathing and circulation. The body responds by attempting to eliminate carbon dioxide from the blood through hyperventilation, characterized by rapid and shallow breathing. Since the concentration of carbon dioxide is so high in the inhaled air, the blood's pH level cannot be normalized. This leads to the release of stress hormones that stimulate receptors in the heart and blood vessels, resulting in an elevated heart rate and increased blood pressure. As a consequence, peripheral blood vessels dilate, leading to lung haemorrhages, pulmonary oedema, and brain haemorrhages, ultimately resulting in the death of the mouse.

[15] 12 ch. 14 § SJVFS 2019:9.

Practical aspects

Carbon dioxide has a higher density than air and is neither flammable nor explosive, making it easy and safe to handle. Compared to many other methods of euthanasia, carbon dioxide is a cheaper alternative. The carbon dioxide is delivered in gas tubes with a high pressure. Through a pressure regulator, gas lines and a flow meter, the gas can be directed into the chamber. The chamber should be designed in such a way that the mice cannot injure themselves and can be visually monitored during killing. The size, density, design and interior of the chamber, and the number of mice in the chamber, can affect how the chamber is filled with gas. This, in turn, may affect how quickly the mice become unconscious. Automatic systems are available that can supply carbon dioxide to individual cages or multiple cages simultaneously using cabinets or connection to a complete IVC system. The space should be gradually filled with carbon dioxide until the carbon dioxide constitutes 80% of the air inside the chamber or cage with mice.

Recommendations

Whenever possible, a high gas pressure into the chamber should be avoided as it can cause cold drafts and loud noises that can stress the mice. In cases of injury, illness, or stress, the respiration of mice may be compromised. When mice exhibit impaired breathing, it is crucial to administer appropriate anaesthesia prior to euthanasia with carbon dioxide or consider alternative humane euthanasia methods. Avoid mixing mice from different cages and always use the home cage whenever possible to reduce stress. If a special gassing-chamber is used, it must be emptied of carbon dioxide between killings. Maintain the flow of carbon dioxide for one minute after the mice take their last breath to ensure that respiration has completely ceased. Remember that carbon dioxide is a heavy gas and that opening the lid is not enough to vent it out. The carbon dioxide needs to be poured out on a well-ventilated surface.

Experiences from Swedish animal facilities

- We have written instructions that are easy to follow. The instruction includes both the process of euthanasia itself and how the function of the automatic system is ensured before euthanasia. The instruction promotes that everyone carries out the process in the same way and feels safe in the execution, which means that everyone can help with the euthanasia.
- I think it is good to have an automatic system that is programmed so that the flow rate is pre-set and does not need to be regulated manually.
- We always euthanize mice in their home cage to avoid stress.
- When there are several mice in the same cage where not all are to be euthanized, we await the next cage change. When changing cages, we let the mice to be euthanized remain in the old home cage and move the other mice to a new cage. The mice to be euthanized remains in their home cage in which they feel safe.

- We regularly check that all equipment is in good condition and that it is working properly.
- I like to have suitable enrichment in the cage during the euthanasia.
- If the mice are not euthanized in their home cage, we clean the space in which they are euthanized so that they do not smell the odour of other mice that have been frightened.
- When the mice are not euthanized in their home cage, I cover the bottom of the space with, for example, paper so that the mice do not get wet or soiled by urine and faeces.

Fatal blow to the head

General information

- The method may only be used for a smaller number of animals.[16]
- The method is approved for mice at all ages.[17]
- The method requires that the mice are being handled.
- The method requires a lot of training and a good knowledge of the technique.

The method in brief

A powerful well-aimed blow to the back of the mouse's head causes immediate unconsciousness and death.

Animal welfare

A fatal blow to the head is a method that quickly leads to unconsciousness and death when performed correctly. There is a risk of incorrect execution which may be associated with suffering. A good technique is crucial for maintaining good animal welfare. To perform a forceful blow to the head of awake mice, they must first be captured and fixed, which may be associated with stress. If there are other mice nearby, they might be affected in a negative way by noises and pheromones from stressed mice.

Physiology

A powerful, well-aimed blow to the back of the head causes a concussion with subsequent brain damage. The blow causes damage to the skull and nearby tissues, whereupon the central nervous system ceases to function. The method leads to immediate unconsciousness and death as normal brain functions cease.

Practical aspects

There are several techniques for performing the method, one of which is to place the mouse on a surface, such as a grid, where it can grip and hold on to the surface. The mouse is then held by the tail and hit hard on the head behind the ears with a hard stick or similar object. No special equipment is required to perform the method and it can thus be performed at a low cost. The method requires high concentration and good accuracy on the part of the person performing the euthanasia and is only permitted for a small number of mice.

Recommendations

Knowledge and skill in using this method is of utmost importance. Regular training and follow-up are required to ensure that the correct technique is used. Physical

[16] 12 ch. 18 § SJVFS 2019:9.

[17] 12 ch. 18 § SJVFS 2019:9.

methods for euthanasia such as a fatal blow to the head can have a greater negative psychological impact on the person executing the euthanasia compared to methods that are not physical. It is important that the persons performing the method feels comfortable with the method and with euthanasia, to ensure both their own mental well-being and the welfare of the mice. When this method is used in awake mice, handling is a potential stress factor. Stress levels can be minimized by handling mice gently and avoiding the use of the tail for capturing or lifting of the animal.

Experiences from Swedish animal facilities

The Swedish 3Rs Center has not received any comments from Swedish animal facilities regarding euthanasia using a fatal blow to the head.

Anaesthetics

General information regarding anaesthesia using inhalation agents

- The method is approved for mice at all ages.[18]
- The method does not require fixation.
- Anaesthesia and euthanasia can be performed in the home cage.
- Several animals can be euthanized at the same time.
- When anaesthetics are used as a method of euthanasia, it takes a long time before the mice die and it may, therefore, be relevant to use additional methods of euthanasia.
- The method may give rise to different types of post mortem changes depending on the preparation used.

General information regarding anaesthesia using injection agents

- The method is approved for mice at all ages.[19]
- The method requires that the mice are being handled.
- The method is best suited for euthanasia of a small number of animals.
- The method may give rise to different types of post mortem changes depending on the preparation used.

The method in brief

An overdose of anaesthetics causes respiratory depression and circulatory failure, leading to unconsciousness and death.

Animal welfare

Inhalation agents

Research has shown that mice, to varying degree, experience several inhalation agents as unpleasant. The fact that inhalation of anaesthetic agents can be experienced as unpleasant does not necessarily mean that they cause pain, but it does mean that the mice avoid the gas in most cases. Mice previously exposed to inhalants may react negatively and become more stressed when exposed repeatedly. The discomfort is likely to increase if the animals in addition are

[18] Table 1, 12 ch. 8 § SJVFS 2019:9.

[19] Table 1, 12 ch. 8 § SJVFS 2019:9.

experiencing transportation, unfamiliar surroundings or regrouping. These factors should, therefore, be avoided as far as possible.

Injection agents

For injection, the mouse must first be captured and fixated, which may be associated with stress. Some injection agents can cause pain depending on how it is injected. Therefore, it is crucial to possess a thorough understanding of the specific injection agent being used and its potential side effects. An incorrectly performed injection can cause suffering to the animal.

Physiology

Use of anaesthetics leads to unconsciousness by affecting the central nervous system. In case of an overdose, unconsciousness is irreversible. Initially, breathing is inhibited and eventually stops completely, leading to circulatory failure and death. Anaesthetics can give rise to different types of post mortem changes depending on which preparation is used.

Practical aspects

Inhalation agents

The Swedish Work Environment Authority prescribes maximum exposure limits for staff working with anaesthetic gases.[20] The Swedish Work Environment Authority also prescribes requirements for documentation, risk assessment and protective measures when working with anaesthetic gases.[21] Among other things, there must be an exhaust that removes excess gas.[22] To perform the method, carburettors, gas lines, carrier gas (such as air or oxygen), and a chamber or mask are usually needed. The size, tightness, design and interior design of the chamber, and the number of mice in the chamber, will affect how the chamber is filled with gas, which in turn affects how quickly the mice become unconscious. If anaesthetic gas alone is used, several minutes of exposure are required to euthanise a mouse. Hence, it might be appropriate to employ an additional euthanasia method once the animal is sufficiently anesthetized. If an alternative method is chosen, it's imperative to ensure the animal is unconscious before euthanasia. To confirm unconsciousness, the pain response can be assessed. One approach is to test the avoidance reflex by gently pinching a hind paw. If the mouse is truly unconscious, it will exhibit no reflexive movement to withdraw its leg. This reflex test should be conducted shortly before administering euthanasia by another method, as the depth of anaesthesia may fluctuate over time. Focus on the hind paws is recommended, as studies suggest that reflexes in the hind legs provide more reliable indicators of consciousness than those in the front legs.

[20] AFS 2018:1.

[21] 7–8 ch. and 10 ch. 3–5 §§ AFS 2023:10.

[22] 10 ch. 4 § AFS 2023:10.

Injection agents

Anaesthetics classified as narcotic drugs must be stored in a way that ensures that no unauthorized person can get access to them[23], and all events that involve a change in the possession must be recorded.[24] Injection agents and euthanized mice must be handled with care to avoid risk to humans, animals and the environment. Injection of an anaesthetic agent can be a technically challenging method that requires more experience and practice compared to the use of anaesthetic gas. Injection agents are best suited for euthanasia of single mice. When a larger number of mice are to be euthanized, the method can be time-consuming and costly. It may also be difficult to maintain the level of concentration required to perform repeated injections over a longer period of time.

Recommendations

Inhalation agents

Use the home cage of the mouse whenever possible. Avoid a high gas pressure into the chamber as it can cause cold drafts and loud noises that can stress the mice. Anaesthetic gas may be used in combination with another method of euthanasia when the depth of anaesthesia is sufficient. In case of injury, illness or stress, breathing may be affected. For mice whose breathing is affected, another method of euthanasia should be considered. When euthanizing neonatal mice, it may be relevant to consider another method of euthanasia, as these mice can hold their breath and survive oxygen deprivation for a long period of time.

Injection agents

Several factors can cause unnecessary discomfort during an injection. Volume, injection site, method of administration, temperature of the injection agent, injection speed, intake of food and water before the injection, as well as the size of the cannula are factors that may have an impact on the welfare of the mouse. When euthanized by this method, handling and fixation before euthanasia can be strong stressors for mice. Stress levels can be minimized by handling mice gently and avoiding the use of the tail for capturing or lifting of the animal.

Experiences from Swedish animal facilities

- We anesthetize mice with Isoflurane. When the mouse is sufficiently anesthetized, the abdomen and thorax are cut open. We take blood samples, collect organs relevant for the study and remove the heart. If a mouse, not included in any study, is to be euthanized, we anesthetize with Isoflurane and then cut open the thorax and remove the heart to ensure death. We always leave the mask with Isoflurane on throughout the procedure, even during retroorbital blood sampling.

[23] 28 § LVFS 2011:9.

[24] 16–23 §§ LVFS 2011:9.

- We first anesthetize with Isoflurane, then perform cervical dislocation on the anesthetized mouse. To ensure death, we check that rigor mortis has set in.
- We anesthetize with Isoflurane, and only when the animal is bled (via the heart, major artery or another large blood vessel) and death is assured, is it removed from the mask.
- Within our organization, the method of putting mice to sleep with anaesthetics, opening the chest and cutting the heart is included in a practical training where everyone needs to have an approved result before they are given the opportunity to work single-handed with the animals.
- I make sure that the area where the mice are anesthetized is clean. Mice can sense if other frightened mice have been present and I want to make sure to keep the stress to a minimum.
- I always check the euthanasia station before bringing mice for euthanasia. The mice should not have to sit and wait unnecessarily if, for example, the Isoflurane needs to be refilled or if any anaesthetic tubing needs to be changed. I want the time to be as short as possible between collection of the animals and the moment when anaesthesia and euthanasia begin.
- I think it is important to be aware that different behaviours can appear depending on the genotype of the mouse. For example, some strains become more stressed when Isoflurane is put on and thus jump around more in the cage, while others take longer time to fall asleep and need to be anesthetized for a little longer.
- I always tip the cage slightly to one side before switching from Isoflurane to carbon dioxide, to ensure the mouse is anesthetized properly.
- When euthanizing with an anaesthetic injection, I use pentobarbital. The mice often fall asleep quietly and quickly, which feels good. The dosage is important, it does not feel good if the mouse has to receive another dose because the first one was not sufficient for euthanasia.
- I put mice to sleep in one lab and perfuse or dissect in another lab, to make sure that the mice that are still awake are not in the room where I perform perfusion and dissection.
- I make sure to collect and prepare all equipment and drugs before euthanasia begins.
- We train and handle the mice carefully throughout their stay with us. In all handling, we use a Vetbed from start to finish. The mice recognize the Vetbed and can feel comfort in having it present. All mice are euthanized one by one, the mice that are waiting are kept in a separate room. When we are going to euthanize with Isoflurane, we habituate and train the mice by placing the nozzle on their nose so that they recognize the procedure when it is time for euthanasia. When anesthetized, the mouse is held in the Vetbed, the nozzle is held above the head and we let the heavy gas fall towards the mouse. We do

this because the mice can become stressed by placing the nozzle directly against the nose, the smell of Isoflurane is also strong. The nozzle is placed tightly against the nose only when the mouse has started to fall asleep. When mice are anesthetized in a box, we make sure the box is empty of gas before placing the mice in it. We believe that anaesthesia is induced in a calmer way if the gas is filled gradually. We cover the box with fabric or the like to calm the mice down, the Vetbed can be included in the bottom of the box to provide comfort for the mice. When we use pentobarbital, it is given either as an intraperitoneal injection or as an intravenous injection into the tail. The mice fall asleep the fastest from the intravenous injection in the tail. When performing injections, the Vetbed is present to provide comfort.

Control methods

General information

When euthanasia has been executed, a control method should always be applied to ensure that the mouse is dead before further action is taken. This can be done by confirming that circulation has stopped permanently or that rigor mortis has set in, provided there is no risk of suffering. When neither cessation of circulation nor rigor mortis can be used, death can be ensured by destruction of the brain, exsanguination, or cervical dislocation.[25]

Control methods in brief

Confirmation of permanent cessation of circulation

Some methods of euthanasia can lead to permanent cessation of circulation, such as decapitation and exsanguination of unconscious mice. Cessation of circulation can also be ensured by cutting open the chest and destroying the heart, other life-sustaining organs, or the central blood vessels such as the large carotid artery. By monitoring over a longer period of time that the animals are not breathing and that they have no pulse, it can be established that these functions have ceased permanently.

Rigor mortis

After death, the musculature stiffens, so-called rigor mortis. The stiffness is due to the lack of oxygen in the muscle cells and the fact that the proteins actin and myosin in the muscles are bound together. By ensuring that rigor mortis has set in, death can be ensured. This method is only permitted if the mice are not at risk of suffering. Waiting for rigor mortis can be time consuming. How quickly rigor mortis occurs and how long it lasts depends on several factors such as temperature in the room, and the age, physical activity, muscle mass and possible pathologies of the mouse. Research has shown that rigor mortis can occur in the jaw joint one hour after death at an indoor temperature of 21ºC ± 1ºC. However, it may take up to three hours before detectable rigor mortis has occurred in the limbs.

Destruction of the brain

The method is based on manual destruction of the animal brain after euthanasia. Destruction can be done with the help of a narrow and sharp instrument that is inserted and rotated in the brain through the opening between the skull and the cervical spine. The instrument must first be inserted forward towards the brainstem and then backwards towards the spinal cord. This method is rarely used in mice.

[25] 12 ch. 7 § SJVFS 2019:9.

Exsanguination

The method involves opening the carotid arteries (uncommon method in mice) or at least one of the central blood vessels such as the large body artery using, for example, scissors or a scalpel.

For more information, see page 8.

Cervical dislocation

There are several established techniques for performing cervical dislocation, but the basic principle is the same. Cervical dislocation is a manual method that involves initial fixation of the mouse with a firm grip at the base of the tail and firm pressure on the neck, just behind the skull. When the mouse is fixated, the neck is dislocated with a definite movement that push down and forward on the neck, while stretching the spine with a controlled pull on the root of the tail. There is a risk of unsuccessful execution, which may cause suffering to the animal. Cervical dislocation performed solely through tail jerking, without adequate pressure applied to the neck, increases the risk of fracturing the spinal cord inaccurately. If an incorrect dislocation is performed on unconscious mice, there is a risk that they will wake up to pain and suffering.

For more information, see page 12.

Recommendations

Choose a control method that you are comfortable with and that complements the method of euthanasia that you have chosen. Be prepared to execute a fast euthanasia technique if the mouse is still alive when checked.

Experiences from Swedish animal facilities

- Organ removal and blood sampling is our most common procedure at termination of studies. Thus, exsanguination with removal of vital organs is our most common method of ensuring death.
- We check that rigor mortis has set in on mice which we have anesthetized and performed dislocation of the neck.
- I cut the diaphragm and heart open, it is very definitive and there is no risk of the mouse waking up again.
- The standard method of euthanasia used by our animal technicians includes cutting of the diaphragm and heart to ensure death, so we do this on all animals that are being euthanized. The method is good as it is safe, but quite time-consuming when many animals are being euthanized in a day.
- I think cervical dislocation feels like an unsafe method to use to ensure death. If I were to make a mistake, I may not notice that the mouse is still alive if it is deeply unconscious. When using cervical dislocation, I supplement with an additional method ensuring that circulation has ceased.

- I let the mice lie for a long time after euthanasia and always check that rigor mortis has set in to make sure they have died. While I wait, I perform other tasks in the same room.
- When euthanized by anaesthetic overdose, we continue to observe the mice for some extra time after the circulation has stopped, to thoroughly confirm death before we put them in the freezer. In this way, we minimize the risk of animals waking up in the freezer.
- I check that rigor mortis has set in by placing the mice lined up in a cage while I work on other things in the same room. Since I know exactly how they were placed originally, I can easily see if they have changed position.

Please contact us at 3Rcenter@jordbruksverket.se if you have additional suggestions for this document or ideas about something that you think the Swedish 3Rs Center should work with.

References

Introduction

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Boivin, G.P., Bottomley, M.A., Schiml, P.A., Goss, L. & Grobe, N. (2017). Physiologic, Behavioral, and Histologic Responses to Various Euthanasia Methods in C57BL/6NTac Male Mice. *Journal of the American Association for Laboratory Animal Science* (56): 69-78.

Charbonneau, R., Niel, L., Olfert, E., von Keyserlingk, M. & Griffin, G. (2010). CCAC guidelines on: euthanasia of animals used in science. https://ccac.ca/en/guidelines-and-policies/the-guidelines/types-of-animal-guidelines.html (2023-10-02).

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050 (2023-10-02).

Hurst, J.L. & West, R.S. (2010). Taming anxiety in laboratory mice. *Nature Methods* 7(10): 825-826.

Marquardt, N., Feja, M., Hünigen, H., Plendl, J., Menken, L., Fink, H. & Bert, B. (2018). Euthanasia of laboratory mice: Are isoflurane and sevoflurane real alternatives to carbon dioxide? *PLoS ONE* 13(9): e0203793. https://doi.org/10.1371/journal.pone.0203793.

North American 3Rs Collaborative. (2023). *Compassion Fatigue Resiliency.* https://www.na3rsc.org/compassion-fatigue/ (2023-06-29).

SJVFS 2019:8. *Statens jordbruksverks föreskrifter och allmänna råd om slakt och annan avlivning av djur.* Regulations by the Swedish Board of Agriculture, in Swedish.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Exsanguinations of unconscious animals

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Chaudry, I.H., Wang, P., Singh, G., Hauptman, J.G. & Ayala, A. (1993). Rat and Mouse Models of Hypovolemic-Traumatic Shock. I Schlag, G., Redl, H. (red.). *Pathophysiology of Shock, Sepsis, and Organ Failure*. Springer, Berlin, Heidelberg. 371-372. https://doi.org/10.1007/978-3-642-76736-4_28.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Decapitation

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Clarkson, M.J., Martin, E.J. & McKeegan, D.E.F. (2022). A review of methods used to kill laboratory rodents: issues and opportunities. *Laboratory Animals* 56(5): 419-436. doi: 10.1177/00236772221097472.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

Hurst, J.L. & West, R.S. (2010). Taming anxiety in laboratory mice. *Nature Methods* 7(10): 825-826.

Iwarsson, K. & Rehbinder, C. (1993). A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post mortem findings. *Scandinavian Journal of Laboratory Animal Science* 20(4):191-205. https://doi.org/10.23675/sjlas.v20i4.738.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Cervical dislocation

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Carbone, L., Carbone, E.T., Yi, E.M., Bauer, D.B., Lindstrom, K.A., Parker, J.M., Austin, J.A., Seo, Y., Gandhi, A.D. & Wilkerson, J.D. (2012). Assessing

Cervical Dislocation as a Humane Euthanasia Method in Mice. *Journal of the American Association for Laboratory Animal Science* 51(3): 352-356.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

Hurst, J.L. & West, R.S. (2010). Taming anxiety in laboratory mice. *Nature Methods* 7(10): 825-826.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Inert gases

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Burkholder, TH., Niel, L., Weed, JL., Brinster, L.R., Bacher, J.D. & Foltz, C.J.S. (2010). Comparison of carbon dioxide and argon euthanasia: effects on behavior, heart rate, and respiratory lesions in rats. *Journal of the American Association for Laboratory Animal Science* 49(4): 448–453.

Detotto, C., Isler, S., Wehrle, M., Vyssotski, A.L., Bettschart-Wolfensberger, R. & Gent, T.C. (2019). Nitrogen gas produces less behavioural and neurophysiological excitation than carbon dioxide in mice undergoing euthanasia. *PLoS ONE* 14(1): e0210818. https://doi.org/10.1371/journal.pone.0210818.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

Leach, M.C., Bowell, V.A., Allan, T.F. & Morton, D.B. (2002). Aversion to Gaseous Euthanasia Agents in Rats and Mice. *Comparative Medicine* 52(3): 249-257.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Injection of chemicals in unconscious animals

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Navarro, K.L., Huss, M., Smith, J.C., Sharp, P., Marx, J.O. & Pacharinsak, C. (2021). Mouse Anesthesia: The Art and Science. *ILAR Journal* 62(1-2): 238-273.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Carbon dioxide

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Boivin, G.P., Bottomley, M.A., Schiml, P.A., Goss, L. & Grobe, N. (2017). Physiologic, Behavioral, and Histologic Responses to Various Euthanasia Methods in C57BL/6NTac Male Mice. *Journal of the American Association for Laboratory Animal Science* (56): 69-78.

Creamer-Hente, M.A., Lao, F.K., Dragos, Z.P. & Waterman, L.L. (2018). Sex- and Strain-related Differences in the Stress Response of Mice to CO Euthanasia. *Journal of the American Association for Laboratory Animal Science* 57(5): 513-519.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

Iwarsson, K. & Rehbinder, C. (1993). A study of different euthanasia techniques in guinea pigs, rats and mice. Animal response and post mortem findings. *Scandinavian Journal of Laboratory Animal Science* 20(4):191-205. https://doi.org/10.23675/sjlas.v20i4.738

Marquardt, N., Feja, M., Hünigen, H., Plendl, J., Menken, L., Fink, H. & Bert, B. (2018). Euthanasia of laboratory mice: Are isoflurane and sevoflurane real alternatives to carbon dioxide? *PLoS ONE* 13(9): e0203793. https://doi.org/10.1371/journal.pone.0203793

Moody, C.M., Chua, B. & Weary, D.M. (2014). The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. *Laboratory Animals* 48(4): 298–304. doi: 10.1177/0023677214546509.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Quagliato, L., Freire, R. & Nardi, A. (2018). The role of acid-sensitive ion channels in panic disorder: a systematic review of animal studies and meta-analysis

of human studies. *Translational Psychiatry* 8(1): 185. doi: 10.1038/s41398-018-0238-z.

Ziemann, A.E., Allen, J.E., Dahdaleh, N.S., Drebot, I.I., Coryell, M.W., Wunsch, A.M., Lynch, C.M., Faraci, F.M., Howard, M.A. 3rd., Welsh, M.J. & Wemmie, J.A. The amygdala is a chemosensor that detects carbon dioxide and acidosis to elicit fear behavior. *Cell* 139(5):1012-21. doi: 10.1016/j.cell.2009.10.029.

Fatal blow to the head

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Clarkson, M.J., Martin, E.J. & McKeegan, D.E.F. (2022). A review of methods used to kill laboratory rodents: issues and opportunities. *Laboratory Animals* 56(5): 419-436. doi: 10.1177/00236772221097472.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

Hurst, J.L. & West, R.S. (2010). Taming anxiety in laboratory mice. *Nature Methods* 7(10): 825-826.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Universities Federation for Animal Welfare. (1972). The UFAW Handbook on the Care and Management of Laboratory Animals. 4 uppl. Longman Group limited.

Anaesthetics

AFS 2018:1. *Arbetsmiljöverkets föreskrifter och allmänna råd om hygieniska gränsvärden.* Regulations by the Swedish Work Environment Authority, in Swedish.

AFS 2023:10. *Arbetsmiljöverkets föreskrifter och allmänna råd om risker i arbetsmiljön.* Regulations by the Swedish Work Environment Authority, in Swedish.

American Veterinary Medical Association (2020). AVMA Guidelines for the Euthanasia of Animals: 2020 Edition. https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf (2023-10-02).

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. Animals 6(9): 50. doi: 10.3390/ani6090050.

Hurst, J.L. & West, R.S. (2010). Taming anxiety in laboratory mice. Nature Methods 7(10): 825-826.

Leach, M.C., Bowell, V.A., Allan, T.F. & Morton, D.B. (2002). Degrees of aversion shown by rats and mice to different concentrations of inhalational anaesthetics. Veterinary Record 150(26): 808-815. doi: 10.1136/vr.150.26.808.

Laferriere, C.A. & Pang, D.S. (2020). Review of Intraperitoneal Injection of Sodium Pentobarbital as a Method of Euthanasia in Laboratory Rodents. Journal of the American Association for Laboratory Animal Science. 59(3): 254-263. doi: 10.30802/AALAS-JAALAS-19-000081.

LVFS 2011:9. *Läkemedelsverkets föreskrifter om kontroll av narkotika*. Regulations by the Swedish Medical Products Agency, in Swedish.

Marquardt, N., Feja, M., Hünigen, H., Plendl, J., Menken, L., Fink, H. & Bert, B. (2018). Euthanasia of laboratory mice: Are isoflurane and sevoflurane real alternatives to carbon dioxide? PLoS ONE 13(9): e0203793. https://doi.org/10.1371/journal.pone.0203793.

Navarro, K.L., Huss, M., Smith, J.C., Sharp, P., Marx, J.O. & Pacharinsak, C. (2021). Mouse Anesthesia: The Art and Science. ILAR Journal 62(1-2): 238-273.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

Son, Y. (2010). Molecular mechanisms of general anesthesia. Korean Journal of Anesthesiology 59(1): 3-8. doi: 10.4097/kjae.2010.59.1.3.

Turner, P.V., Brabb, T., Pekow, C. & Vasbinder, M.A. (2011). Administration of substances to laboratory animals: routes of administration and factors to consider. Journal of the American Association for Laboratory Animal Science. 50(5): 600-613.

Control methods

Capas-Peneda, S., Gonçalves-Monteiro, S., Oliveira, B. & Duarte-Araújo, M. (2016). How do you tell how long has a mouse been dead? Rigor mortis as a tool to estimate mice time of death (TOD) in animal house facilities [Poster]. *FELASA Congress*, 13-16 Juni, Bryssel, Belgium. doi: 10.13140/RG.2.2.15399.09127

Carbone, L., Carbone, E.T., Yi, E.M., Bauer, D.B., Lindstrom, K.A., Parker, J.M., Austin, J.A., Seo, Y., Gandhi, A.D., & Wilkerson, J.D. (2012). Assessing Cervical

Dislocation as a Humane Euthanasia Method in Mice. *Journal of the American Association for Laboratory Animal Science* 51(3): 352-356.

Hawkins, P., Prescott, M.J., Carbone, L., Dennison, N., Johnson, C., Makowska, J., Marquardt, N., Readman, G., Weary, D.M. & Golledge, H.D.R. (2016). A Good Death? Report of the Second Newcastle Meeting on Laboratory Animal Euthanasia. *Animals* 6(9): 50. doi: 10.3390/ani6090050.

SJVFS 2019:9. *Statens jordbruksverks föreskrifter och allmänna råd om försöksdjur*. Regulations by the Swedish Board of Agriculture, in Swedish.

The Swedish 3Rs Center
551 82 Jönköping
Telephone 0771-223 223
3Rcenter@jordbruksverket.se
www.jordbruksverket.se/3R