Technical information animal experimentation

Proficient and animal welfare-compliant euthanasia of laboratory animals 3.01

Mouse, rat, hamster, guinea pig, rabbit, zebrafish, clawed frog

1 Objective

This technical information is addressed to the authorities responsible for animal experiments, their advisory committees and all persons concerned with, carrying out or having responsibility for animal experiments.

The careful handling and competent euthanasia of laboratory animals continues the careful handling of each animal used for experiments right through until its death. It represents an effective contribution to animal welfare and helps to minimise strain on the animals. The continuous improvement of euthanasia methods is a key requirement in the implementation of the 3R principles.

This document explains where information on careful, competent euthanasia of laboratory animals is available, and which euthanasia methods are admissible, conditionally admissible and inadmissible.

Not all euthanasia methods listed in this document meet the definition of euthanasia (section 4). For the sake of simplicity, however, this document uses only the term “euthanasia”.

Version of 8.9.2020, editorial modifications 2. March 2022

BLV-D-8E893401/213

2 Legal bases

2.1 The killing of animals in a way that causes suffering is prohibited (Art. 16 para. 1 lett. a of the Tierschutzgesetz Animal Welfare Ordinance, AWO)

2.2 A vertebrate animal must only be euthanised after it has been rendered unconscious. If stunning is not possible, all measures must be taken to reduce pain, suffering and fear to a minimum (Art. 178 para. 1 of the Tierschutzverordnung (Animal Welfare Ordinance, AWO).

2.3 No person must subject an animal to pain, suffering, harm or fear without justification, or otherwise violate its dignity (Art. 4 para. 2 AWA). Pain, suffering or harm may be inflicted on an animal only if this is unavoidable for the intended purpose (Art. 20 para. 1 AWA).

2.4 Vertebrates and decapods may only be euthanised by competent persons (Art. 177 AWO). A competent person is a person who has acquired the necessary knowledge and practical experience of animal euthanasia under expert guidance and supervision, and who euthanises animals regularly.

2.5 Animal experiments which inflict pain, suffering or harm on the animal, induce anxiety in the animal, substantially impair its general well-being or disregard its dignity in any other way must be limited to the indispensable minimum (Art. 17 AWA).

2.6 If an animal continues to show pain after an experimental procedure, it must be painlessly euthanised as soon as the objective of the experiment permits (Art. 135 para. 7 AWO).

2.7 Animals must not be euthanised in rooms where animals are housed (Art. 135 para. 9 AWO).

3 Principles and responsibilities

3.1 Euthanasia is an integral part of the animal experiment.

3.2 Only best-practice euthanasia methods compliant with animal welfare should be used (admissible euthanasia methods, Section 4). The approval of conditionally admissible euthanasia methods (Section 5) requires a reasoned justification. No exceptions are granted for inadmissible euthanasia methods (Section 7).

3.3 Non-use of a euthanasia method allowing to reduce the constraint on the animals to a minimum may result in the experiment being assigned a higher severity degree. This is taken into account in the weighing of interests.

3.4 Specific knowledge of euthanasia is required. Various forms of training and information are available for an introduction to the topic and for continuing education. Knowledge must be continuously expanded and kept up to date. A selection of training and information opportunities and relevant publications is given in Section 8.

3.5 Particular attention should be paid to the following points:

It is essential to avoid inducing anxiety or exposing animals to stressful situations. If this is unavoidable, the situation causing the constraint must be kept as short as possible. Preference should therefore be given to methods which require no or only short and gentle restraint of the animals. Possible defensive reactions, aggression and agitation can thus be avoided or at least reduced.

Long transfers to the place of euthanasia should be avoided. These can cause the animals more stress than the euthanasia itself.

Animals must continue to be monitored during euthanasia to allow immediate intervention if necessary. The visibility of all animals in the cage or container must not be obstructed.

Animals must not be disposed of until death has been confirmed. Death must be confirmed in each individual animal according to the reliable signs of death. Death must also be confirmed in anaesthetised animals before they can be disposed of, if necessary by means of an additional measure (Section 6)

3.6 Anaesthesiology also includes euthanasia. The experts in anaesthesia and analgesia (ECVAA[1], ACVAA[2]) are excellent contacts for euthanasia issues, as are the experts in laboratory animal medicine (ECLAM[3], ACLAM[4]). Ideally, they should be consulted as early as the experiment planning stage.

3.7 Study directors are responsible for the best possible selection, planning and conduct of euthanasia, and for the qualifications of persons carrying out euthanasia.

3.8 Even a welfare-compliant euthanasia method may cause constraint on the animals if poorly performed. It should therefore be practised in the presence of an experienced person.

3.9 The euthanasia of animals must be monitored by the authorities (methods, manner and means of performance, handling of animals, visibility and possibility to observe the animals in euthanasia chambers and cages, equipment, premises, level of training of persons carrying out euthanasia).

[1] ECVAA – European College of Veterinary Anaesthesia and Analgesia
[2] ACVAA – American College of Veterinary Anesthesia and Analgesia
[3] ECLAM – European College of Laboratory Animal Medicine
[4] ACLAM – American College of Laboratory Animal Medicine

4 Admissible euthanasia methods

Admissible methods ensure that the animals are unconscious when they are euthanised. These methods meet the definition of “euthanasia”. This category includes euthanasia by pentobarbital overdose and euthanasia of animals under general anaesthesia.

4.1 Pentobarbital is the anaesthetic of choice for the euthanasia of mice, rats, hamsters, guinea pigs, rabbits and clawed frog.

For dosages, consult specialist publications or experts.

Pentobarbital may need to be diluted for administration, in order to reduce viscosity and for exact dosing of the anaesthetic.

It is essential to ensure that the animals have reached a surgical depth of anaesthesia before they are bled (exsanguinated), perfused or have organs removed.

In mice, rats, hamsters, guinea pigs, clawed frogs and in rabbits up to two weeks old pentobarbital is to be injected intraperitoneally or into the coelom.

In rabbits over two weeks old, pentobarbital should be administered only intravenously.

Mouse	Pentobarbital
Rat	
Hamster	
Guinea pig	
Rabbit	
Clawed frog	

4.2 Inhalation or injection anaesthetics may be used for general anaesthesia before euthanising mice, rats, hamsters, guinea pigs or rabbits. If animals are under general anaesthesia before euthanasia, euthanasia methods not permitted in conscious animals may also be used (e.g. decapitation, perfusion, bilateral pneumothorax). Death must occur under surgical depth of anaesthesia and must be confirmed.

For dosages, consult specialist publications or experts.

These anaesthetics may need to be diluted to ensure precise dosing.

It is essential to ensure that the animals have reached a surgical depth of anaesthesia before they are bled (exsanguinated) or perfused.

Mouse	Euthanasia under inhalation or injection anaesthesia
Rat	
Hamster	
Guinea pig	
Rabbit	

5 Conditionally admissible euthanasia methods

These methods also lead to the certain death of the animals. For some euthanasia methods, however, there is a lack of reliable evidence from an animal welfare perspective concerning their effects on animals, especially whether, how and how quickly animals become unconscious. This is especially true for fish. The use of conditionally admissible euthanasia methods should be substantiated. This may mean that the experiment is assigned a higher severity degree. It is taken into account in the weighing of interests.

5.1 Recent findings have raised questions about carbon dioxide (CO_2) from an animal welfare perspective (Hawkins et al 2016, Axiak Flammer et al. 2019, Steiner et al 2019). However, no alternative method is currently available for the euthanasia of large groups of animals.

As an interim solution, the euthanasia of mice and rats using CO_2 is admissible from the age of two weeks.

For CO_2 inhalation, animals are placed in a closed, transparent container. Ideally, they are left in their home cage. Animals must be kept in this container for a sufficient time. The filling rate and exposure time must be species-specific. See publications for recommendations. After removal, each animal must be checked according to the reliable signs of death to make sure death has occurred.

Mouse from 2 weeks	CO_2 (carbon dioxide)
Rat from 2 weeks	

5.2 Decapitation is the severing of the neck between the cranial half of the neck and the head, using a sharp instrument.

Specially developed devices (guillotines) are to be used to ensure that the tissues are severed quickly, correctly and in the right place. Special care must be taken to ensure that the blade cuts through the animal's neck close to the head. In mice up to two weeks old, a quick forceful cut with scissors can be used.

In mice and rats more than two weeks old, decapitation without anaesthesia may be approved only in justified exceptional cases.

Mouse up to 2 weeks	Decapitation without anaesthesia
Rat up to 2 weeks	

5.3 In cervical dislocation (breaking of the neck), the skull and cervical spine are displaced in relation to each other. If carried out correctly, this severs the spinal cord. However, if the nerve pathways are not disrupted quickly and completely, cervical dislocation entails pain for the animal. Tetraplegia, paraplegia and spinal trauma do not produce unconsciousness and rapid death of the animals.

The large blood vessels to the head are not always severed in cervical dislocation. Immediately after cervical dislocation, therefore, animals must be bled (exsanguinated) by opening the large cervical blood vessels. Exsanguination by opening the aorta or other blood vessels that are not accessible without prior surgical intervention is not permitted; nor is opening of the abdomen or chest to remove organs before exsanguination.

In mice and rats up to two weeks old, cervical dislocation without anaesthesia, with exsanguination before any other procedure, may be approved in justified exceptional cases.

Mouse up to 2 weeks	Cervical dislocation without anaesthesia with exsanguination
Rat up to 2 weeks	

5.4 The acceptability from an animal welfare perspective of etomidate, metomidate, tribromoethanol, phenoxyethanol, tricain methanesulfonate (MS222) and ice water for the euthanasia of zebrafish, zebrafish larvae and tadpoles is open. In particular, it is not clear whether and how quickly the animals become unconscious.

Zebrafish	Etomidate, metomidate, tribromoethanol, phenoxyethanol, tricaine methanesulfonate (MS222)
Zebrafish larvae	
Tadpoles	
Zebrafish	Ice water with decapitation

6 Ensuring death

Death of each individual animal must be ensured according to the reliable signs of death, before it is disposed of.

Following an admissible or conditionally admissible euthanasia method, various methods may be used if death must be ensured by a second method. The choice of the method depends on the species. Examples:

Mouse	Decapitation, bleeding (exsanguination), perfusion, bilateral pneumothorax under surgical depth of anaesthesia
Rat	

7 Inadmissible substances, gases, methods

Substances, gases and methods are inadmissible on various grounds:
- admissible or conditionally admissible euthanasia methods are available
- they do not lead to unconsciousness before euthanasia
- death does not occur or does not occur immediately and without pain, suffering or anxiety
- occupational safety aspects need to be taken into account

Ether, chloroform, T61, benzodiazepines, chloral hydrate, α-chloralose, urethane, muscle relaxants, insulin, clove oil, acids, alkalis, osmotically active substances

CO_2 (carbon dioxide) in the hamster, guinea pig, rabbit, zebrafish and clawed frog, and in mice and rats up to two weeks old, CO (carbon monoxide)

Peroral, intramuscular, rectal, retrobulbar and intracerebral administration of anaesthetics; intravascular and intracardiac injection of air

Decapitation of non-anaesthetised mice and rats from two weeks old, hamsters, guinea pigs, rabbits, zebrafish; decapitation of clawed frogs without destruction of the brain and spinal cord

Striking animals against an edge or on the ground, stunning blow

Whole-body microwave irradiation

Administration of electrical current

Exposure of fish to air

Hyperthermia in fish and clawed frogs

Ice water in zebrafish without decapitation, freezing of zebrafish, zebrafish larvae, clawed frogs and tadpoles (e.g. at -20°C, -80°C, -120°C, -180°C and in liquid nitrogen)

8 Training, information, publications

Amendola L and Weary DM - 2020 - Understanding rat emotional responses to CO_2. Transl Psychiatry 10:253 https://doi.org/10.1038/s41398-020-00936-w

Axiak Flammer S et al - 2019 - Alternatives to carbon dioxide - taking responsibility for humanely ending the life of animals. Animals 9:482 https://doi.org/10.3390/ani9080482

AVMA - 2020 - Guidelines for the euthanasia of animals https://www.avma.org/sites/default/files/2020-01/2020-Euthanasia-Final-1-17-20.pdf

Conlee KM et al - 2005 - Carbon dioxide for euthanasia: Concerns regarding pain and distress, with special reference to mice and rats. Lab Anim 39:137-61 http://journals.sagepub.com/doi/pdf/10.1258/0023677053739747

Cressey D - 2012 - Rodent euthanasia methods under scrutiny. Nature Dec 19 http://www.nature.com/news/rodent-euthanasia-methods-under-scrutiny-1.12083

Cressey D - 2013 - Best way to kill lab animals sought. Nature Aug 6 http://www.nature.com/news/best-way-to-kill-lab-animals-sought-1.13509

Cressey D - 2014 - Fish-kill method questioned. Nature Feb 25 http://www.nature.com/news/fish-kill-method-questioned-1.14768

FLAIRE Learning - 2017 - Humane methods of killing laboratory animals https://www.nc3rs.org.uk/euthanasia

Grens K - 2014 - To kill a lab rat. The Scientist Nov 4 http://www.the-scientist.com/?articles.view/articleNo/41378/title/To-Kill-a-Lab-Rat/

Hawkins P et al - 2006 - Newcastle Consensus Meeting of carbon dioxide euthanasia of laboratory animals https://www.nc3rs.org.uk/sites/default/files/documents/Events/First%20Newcastle%20consensus%20meeting%20report.pdf

Hawkins P et al - 2016 - A good death? Report of the second Newcastle Meeting on laboratory animal euthanasia https://www.nc3rs.org.uk/news/report-second-newcastle-meeting-laboratory-animal-euthanasia

Köhler A et al - 2017 - Report of Workshop on euthanasia for zebrafish - a matter of welfare and science. Zebrafish Oct 2 https://www.ncbi.nlm.nih.gov/pubmed/28968196

Lalonde-Robert V et al - 2012 - Electroencephalographic and physiologic changes after tricaine methanesulfonate immersion of African clawed frogs (Xenopus laevis). J Am Assoc Lab Anim Sci 51:622-7 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3447452/pdf/jaalas2012000622.pdf

Matthews M and Varga ZM - 2012 - Anesthesia and euthanasia in zebrafish. ILAR J 53:192-204 https://www.ncbi.nlm.nih.gov/pubmed/23382350

Moody CM et al - 2014 - The effect of carbon dioxide flow rate on the euthanasia of laboratory mice. Lab Anim 48:298-304

NC3Rs - 2013 - Euthanasia https://www.nc3rs.org.uk/euthanasia

Steiner A et al - 2019 - Humanely ending the life of animals: Research priorities to identify alternatives to CO_2. Animals 9:911 https://doi.org/10.3390/ani9110911

Strykowski JL and Schech JM - 2015 - Effectiveness of recommended euthanasia methods in larval Zebrafish (Danio rerio) J Am Assoc Lab Anim Sci 54:81-4 https://www.ncbi.nlm.nih.gov/pubmed/25651096

Torreilles SL et al - 2009 - Evaluation and refinement of euthanasia methods for Xenopus laevis. J Am Assoc Lab Anim Sci 48:512-6 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2755021/pdf/jaalas2009000512.pdf

Turner PV et al - 2020 - Welfare Impact of Carbon Dioxide Euthanasia on Laboratory Mice and Rats: A Systematic Review. Front Vet Sci July 22 https://doi.org/10.3389fvets.2020.00411

Wilson JM et al - 2009 - Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (Danio rerio). J Am Assoc Lab Anim Sci 48:785-9
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2786934/pdf/jaalas2009000785.pdf

Hawk TC et al - 2005 - Formulary for laboratory animals. 3rd edition. Blackwell Publishing
https://norecopa.no/textbase/formulary-for-laboratory-animals

9 Legislation

***Tierschutzgesetz* (Animal Welfare Act, AWA) of 16 December 2005 (SR 455)** As at 1 May 2017

Art. 4 AWA	Principles
Art. 16 AWA	
Art. 20 AWA	Conduct of experiments
Art. 21 AWA	
Art. 26 AWA	Maltreatment of animals
Art. 40 AWA	Supervision by the Federal Government

***Tierschutzverordnung* (Animal Welfare Ordinance, AWO) of 23 April 2008 (SR 455.1)** As at 20 March 2018

Art. 4 AWO	Feeding
Art. 60 AWO	Feed and grooming
Art. 113 AWO	Permitted deviations from the provisions of this ordinance
Art. 132 AWO	Requirements upon study directors
Art. 134 AWO	Requirements upon persons conducting experiments
Art. 135 AWO	Performance of experiment
Art. 137 AWO	Criteria for assessing the essential measure of animal experiments that entail constraint on the animal
Art. 139 AWO	Approval procedure
Art. 177 AWO	Requirements upon persons involved in the euthanasia and slaughter of animals
Art. 178 AWO	Stunning/anaesthesia requirement

FEDERAL FOOD SAFETY AND VETERINARY OFFICE