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## 1 Changes from the previous version

| Date | New version |
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## 2 Technical terms and abbreviations

|       |   |
|-------|---|
| CAF   | Central Animal Facilities                 |
| EAC   | Experimental Animal Center                |
| TSchG | Tierschutzgesetz                          |
| TSchV | Tierschutzverordnung                      |
| AWA   | Animal Welfare Act                        |
| AWO   | Animal Welfare Ordinance                  |
| AVMA  | American Veterinarian Medical Association |
| PI    | Principal Investigators                   |
| LTK1  | Labortierkunde module-1 course            |

## 3 Goals and purposes

This Standard Operating Procedure regulates euthanasia, spontaneous death, and disposal of cadavers. It applies to the Central Animal Facilities of the Experimental Animal Center.

Animals being euthanized should experience minimal pain, fear, or other significant stress prior to their death. The EAC is committed to (1) promote best practices and ensure that pain and distress are minimized in euthanized laboratory animals and (2) assist the scientists by clarifying the specific procedures according to the Swiss legislation.

Euthanasia is conducted according to Swiss regulations (TschG/AWA, TschV/AWO), the technical information 3.01 of the BLV (Sep. 2020), and the Guidelines for Euthanasia of the AVMA (2020 Edition) and the Guide.

- It is essential to avoid inducing anxiety or exposing animals to stressful situations.
- Long transfers to the place of euthanasia should be avoided.
- Animals must continue to be monitored during euthanasia.
- Animals must not be euthanized in rooms where animals are housed (Art. 135 para. 9 AWO)

## 4 Responsibilities

Veterinarians, animal care staff, principal investigator (PI) and their research staff.

Animals may only be euthanized by persons who have successfully completed the LTK 1 course or equivalent recognised course, are accredited and properly trained.

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All PIs must assure that their research staff are duly trained and adhere to the animal care and use protocols, policies, and guidelines.

Training on the use of the equipment and appropriate methods of euthanasia is available from the EAC staff.

## 5 Species

This SOP applies to mice, rats and rabbits.

## 6 Methods

### 6.1 Acceptable methods A; conditionally acceptable CA; Not acceptable NA for Switzerland

| Mouse  | Fetuses and newborns<br>up to 2 days | Up to 2<br>weeks | From 2<br>weeks | From 3<br>weeks |
|--|--------------------------------------|------------------|-----------------|-----------------|
| Decapitation /exsanguination/organ<br>removal/perfusion under anesthesia | A                                    | A                | A               | A               |
| Decapitation without anesthesia  | A                                    | A                | NA              | NA              |
| Cervical dislocation without anesthesia<br>with exsanguination           | A                                    | A                | NA              | NA              |
| CO <sub>2</sub>  | NA                                   | NA               | CA              | CA              |

| Rat  | Fetuses and newborns<br>up to 2 days | Up to 2<br>weeks | From 2<br>weeks |
|--|--------------------------------------|------------------|-----------------|
| Decapitation /exsanguination/organ<br>removal/perfusion under anesthesia | A                                    | A                | A               |
| Decapitation without anesthesia  | A                                    | A                | NA              |
| Cervical dislocation without anesthesia<br>with exsanguination           | A                                    | A                | NA              |
| CO <sub>2</sub>  | NA                                   | NA               | CA              |

| Rabbit           | Up to 2 weeks | From 2<br>weeks |
|------------------|---------------|-----------------|
| Pentobarbital iv | A             | A               |
| Pentobarbital ip | A             | NA              |

### 6.2 Confirmation of death

Death must **always** be confirmed by an approved second technique, like decapitation, exsanguination, perfusion, organ harvesting, bilateral pneumothorax or rigor mortis.

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### 6.3 Pregnant rodents, embryos and foetuses

When foetuses are not required for study, the pregnant animal is euthanized with an acceptable method and the foetuses are left "in utero" for up to 15-20 min after the dam's death; the lack of perfusion and consequent hypoxia will result in painless death of the foetuses. (AVMA 2020).

It is believed that foetuses and embryos are in a state of unconsciousness throughout pregnancy and therefore cannot consciously experience breathlessness or pain associated with dying after euthanasia of the dam.

## 7 Work processes

### 7.1 Euthanasia methods described

- 7.1.1 CO<sub>2</sub> asphyxiation
- 7.1.2 Barbiturate / other injectable / inhalant anaesthetics overdose
- 7.1.3 Physical methods

### 7.2 Procedures

The procedures are defined according to the documents

- 7.2.1 Support Doc. 1 "Rodent CO<sub>2</sub> Euthanasia Guideline".
- 7.2.2 Support Doc. 2 "Barbiturate / other injectable / inhalant anaesthetic overdose Euthanasia Guideline".
- 7.2.3 Support Doc. 3 "Rodent physical method Euthanasia Guideline".

## 8 Procedure in case of spontaneous death in breeding and experiments

Animals in experiments must be accounted with severity degree 3 in the licence. For GMO animals in breeding please refer to the SOP \_distress monitoring.

Cadavers must be removed from the cages and animal room immediately; the animal caretakers will place the cadavers in the refrigerator in labelled plastic bags; these are discarded after 1 week.

- The caretakers inform the veterinarian and the PI per mail. The veterinarian will contact the PI to discuss the possible cause of spontaneous death and decide on the actions and further use of the carcass.
- The scientists inform the veterinarian in case of spontaneous death at [caf@dbmr.unibe.ch](mailto:caf@dbmr.unibe.ch) to discuss the need for further action and investigations.

## 9 Management and storage of documents

### 9.1 Records

Each animal that is euthanized or dies spontaneously must be recorded on the Euthanasia of Animals form AND in the data management system Pyrat.

The Euthanasia of Animals forms are collected regularly by the animal caretakers and archived in the CAF office for at least two years.

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## 10 Disposal of carcasses

The carcasses are disposed of as follows:

- BSL2: The carcasses from the BSL2 are autoclaved weekly together with the contaminated material from the BSL2. After autoclaving, the carcasses are disposed of in the normal collection bin at Mu31.
- Mu31: The collection bucket of Murtenstrasse 31 is placed on the delivery ramp of the CAF on Monday morning at 06:30 am. There it is picked up by the transport service and brought to the collection point of VETSUISSE.
- Mu50: The collection bucket is placed in the disposal room on floor A on Monday morning. There it is picked up by the transport service using an access card and taken to the collection point of VETSUISSE.
- Mu40: The carcass is stored at Murtenstrasse 40 in the refrigerator of the processing plant. On Monday morning, it will be picked up by the animal caretakers and brought to the Mu50 collection bucket, which was previously placed in the disposal room on floor A.
- In VETSUISSE the collected carcasses are stored and brought for incineration in Lyss weekly.

### 10.1.1 Competencies and responsibilities

Disposal of carcasses: the animal caretakers care that the collection buckets are provided in time.

Collection of carcasses: Technische Dienst, Vetsuisse

## 11 References

### 11.1 Legal basis

TSchG (Animal Welfare Act, AWA), TSchV (Animal Welfare Ordinance, AWO),

- 11.1.1 The killing of animals in a way that causes suffering is prohibited (Art. 26 §. 1 AWA).
- 11.1.2 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 §. 1 AWO).
- 11.1.3 No person must subject an animal to pain, suffering, harm or fear without justification, or otherwise violate its dignity (Art. 4 §. 2 AWA). Pain, suffering or harm may be inflicted on an animal only if this is unavoidable for the intended purpose (Art. 20 §. 1 AWA).
- 11.1.4 Vertebrates and decapods may only be euthanised by competent persons (Art. 177 AWO).
- 11.1.5 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 §. 7 AWO).
- 11.1.6 Animals must not be euthanised in rooms where animals are housed (Art. 135 §. 9 AWO).

### 11.2 Technical information and current evidence

- 11.2.1 [Technical information BLV 3.01 \(10.09.2020\)](#)
- 11.2.2 [AVMA Guideline on Euthanasia: 2020 Edition](#)
- 11.2.3 [The Guide, 8<sup>th</sup> Edition](#)

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## 12 Support documents

|                |  |
|----------------|--|
| Support Doc. 1 | "Rodent CO2 Euthanasia Guideline".   |
| Support Doc. 2 | "Barbiturate / other injectable / inhalant anaesthetic overdose Euthanasia Guideline". |
| Support Doc. 3 | "Rodent physical method Guideline".  |
| Support Doc. 4 | "Pentobarbital euthanasia dose rodents".   |