

Standard operasjonsprosedyre: Termination of mice and rats

SOP nr: 8-01

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TERMINATION OF MICE AND RATS

1.0 PURPOSE

- 1.1 To ensure that the termination of animals is done in a humane and safe way.
- 1.2 To ensure that the correct animals are terminated.
- 1.3 To ensure that Science Linker (SL) is updated.

2.0 DIVISION OF RESPONSIBILITY

- 2.1 Everyone who terminates animals is responsible for doing so by law and in a humane way.
- 2.2 KPM will terminate animals in the case of illness or injury or by request in Science Linker.
- 2.3 Everyone who terminates animals must have received training for this prior to termination.
- 2.4 The person completing the termination must remove the animals from Science Linker (SL).

3.0 PROCEDURE

Procedure for termination of animals by employees at the Animal Facility:

- 3.1 Users can create a "Termination" request in Science Linker (SL) when they want KPM to perform terminations. The user must make sure to add the necessary information under "Termination type". All termination requests are prioritized and will be carried out as soon as possible. Requests made before 12pm (noon) will often be performed the same day. Requests made after 12pm will be performed the following day. Exceptions is weekends and red days where these requests will not be performed. With a termination request a fee follows according to our "addition services" on our [homepage](#).
- 3.2 When animals are terminated upon request, a list of the terminations can be printed out from SL then you continue with the termination as followed:
 - Entire cages to be terminated are marked with a big X. you have to terminate these cags the same day.
 - Verify that the cages and the number of animals correspond with the list and SL. Cages containing animals that are all to be terminated, are collected on a trolley.
 - The animals are virtually terminated in SL before they are physically terminated by completing the termination request. This makes it possible to verify that the correct animals are terminated.



- If there are more than one request in the same cage, it could be an advantage to perform that request first (e.g. breeding request).
 - Individual animals to be terminated are terminated together with the other animals or marked with a note on the cage and terminated when time permits. Make sure to update the cage card.
 - Animals are to be physically terminated immediately after they have been removed from SL.
NB! Make sure you control the ear tags and the request carefully before termination.
 - Place the cadavers in a plastic bag (or glove) and tie it shut. Place the plastic bag inside a yellow biohazard bin inside the cadaver freezer. KPM empties the freezer
- 3.3 Upon discovery of sick or injured animal, which are **not in trials**, and where termination is deemed necessary, the animals must be terminated as soon as practically possible (See Appendix 1 and 2). It is considered whether KPM-user should be contacted by telephone or e-mail. Make sure to copy in the Head of Department and PMSK in the correspondence. SL and cage card should be updated.
- 3.4 If the animal is healthy enough to wait the person discovering the animal will inform KPM-user by e-mail before the termination. The e-mail should contain:
- Information regarding the animal in question (the individual is marked in SL and the information about the animal is copied).
 - The condition of the animal.
 - Time and date when the termination will be performed. The time of termination shall take place within a reasonable time limit based solely on the condition of the animal.
 - The head of department and PMSK will be connected.
- 3.5 **Animals in trials** that are detected sick or injured must be scored before euthanasia. Results should be reported immediately, preferably via a telephone conversation to a group leader or employees taking part in the trials. The head of department and PMSK must be connected by mail or phone. Information must be provided about the animal in question, the reason for termination and the time of termination. Animals are valuable especially those in experiments, often it is desirable to harvest organs after termination, **always contact the user before termination of these animals**. Remember to update science linker and cage cards after euthanasia.

Termination performed by KPM-user:

- 3.6 Animals that are terminated during experiments or terminated due to illness or injury can be registered as terminated in SL after the animal has been physically terminated. Users can create a "Termination" request in SL and mark the request with "Physical animals already terminated". Make sure to add the necessary information under "Termination type" and "Termination method".
- 3.7 Registration of "termination type" in Science Linker is described in Appendix 2: Termination of animals in SL.

Termination Methods:

- 3.8 **Cervical dislocation:** Termination can be carried out on mice, and rats under 150 g, provided that thorough training has been given by qualified personnel. Note: When practicing neck dislocation, animals must be anaesthetized or terminated with CO₂.

- 3.9 Make sure the neck is completely dislocated before placing the animal in a plastic bag or glove, tie the bag shut, and throw the cadaver in the yellow hazardous waste-bin inside the cadaver freezer.
- 3.10 When euthanizing with CO₂, the animals must be handled extra gently before termination and transported carefully in their original cage to the lab. It is forbidden to transport mice and rats together to the lab.
- 3.11 It is recommended to euthanize mice/rats in their original cage where the animal has been housed. If that is not possible, put the animals together in one 900 cage. It is allowed to euthanize a maximum of 15 mice or 4-5 (small) rats at a time. The animals should not be left in a foreign place longer than necessary. Start euthanasia right after grouping of animals. Extractor arms should be used where available (attachment 4). P2/P3 mask is recommended. Never allow other animals to observe the euthanasia.


3.12 The procedure for CO₂ euthanasia:



- Remove the cage lid and the food basket, replace the lid with the metal lid connected to the CO₂. There are lids for all GM500 and GM/GR900 cages where the gas can be connected.
- Make sure that the tube conveying the gas into the cage is not blocked by bedding, nest material or similar. Check that the lid is connected to CO₂ and not oxygen.
- First, open the gas outlet from the wall and then open the pressure gauge to approx. 2.5 bar. To start turn the nob on the flowmeter to the bottom line (40). Once the animals are unconscious turn the nob on the flowmeter to the top line (70).
- Within a few minutes, the rats will start to get drowsy and lie down. Mice do not necessarily show the same drowsiness when terminated with CO₂ and may become more active for a while due to the discomfort of elevated CO₂ concentrations in the blood. This can be observed by jumping and eventually gasping reflex even if they are unconscious.
- The animals should be monitored until they are unconscious. After CO₂ gassing, the animals must be removed from the cage and killed with cervical dislocation (mice) or guillotine (rats) when they no longer breathe or react to stimuli. Alternatively, the animals can lie in the cage with the lid on for 20 minutes to ensure that they are choked by the gas if you have no access to guillotine.
- When done, turn off the gas, first the outlet from the wall, then the pressure gauge and finally the flowmeter. The CO₂ pressure-meter nob and the flowmeter nob should not be screwed too tightly as this may damage the equipment and damage calibration.
- Clearly pregnant females should be opened and the offspring should also be terminated.
- Put the cadavers in a plastic bag and tie it shut, place the bag inside a yellow hazardous bin in the freezer.
- Clean the surfaces and metal lid with soapy water and 70% ethanol. Make sure to remove all blood stains with soapy water. Sweep the floor. Used cages are placed on the shelf outside dirty side of the washery.

4.0 HEALTH, SAFETY AND ENVIRONMENT (HSE)

- 4.1 Everyone must be adequately trained to ensure that the proper clothing and protective gear are used.

- 4.2 Everyone who handles animals must have adequate training and practice to ensure that the animals are handled and terminated in the proper way.
- 4.3 The work must be carried out under ventilation to minimize exposure to allergens and potential pathogens and to limit the spread of these to the surrounding areas.
- 4.4 Everyone who handles chemicals must have adequate training and access to proper protective gear in order to ensure the safe use of these chemicals.
- 4.5 Everyone should be familiar with the Eco Archive and Safety Data Sheets for the chemicals they are exposed to (gas under pressure).
- 4.6 Everyone must ensure that the CO₂ gas is correctly switched off before leaving the lab. Carbon dioxide is an odourless gas that is heavier than air. When done with the CO₂, make sure the cage is closed before taking it to the washery. Never attempt to smell the gas.
- 4.7 Make sure there is no leakage from the hose and valves before and after use.

| Chemical | CAS no | Pictogram | Hazard statements | Precautionary statements |
|----------------|----------|---|---|---|
| Carbon dioxide | 124-38-9 |  | H280 Contains gas under pressure; may explode if heated | P403: Store in a well ventilated place. |

| Kemetyl technical ethanol 96% | CAS no | Pictogram | Hazard statements | Precautionary statements |
|-------------------------------|---------|--|---|---|
| Ethanol | 64-17-5 |   | H225 Highly flammable liquid and vapour H319 Causes serious eye irritation | P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. P370 + P378 In case of fire: extinguish with carbon dioxide (CO ₂), foam, powder or water. |

5.0 EQUIPMENT AND MAINTENANCE

- 5.1 GM500 and GM/GR900 cages
- 5.2 Gloves
- 5.3 Soapy water
- 5.4 Paper towels

5.5 70 % Ethanol

5.6 Outlet with CO₂, flowmeter and lid.

6.0 HISTORY OF EDITING

6.1 Transferred to the new template and adapted to the new department 15.11.12.

6.2 26.06.2018 (Frøydis Kilmer)

6.3 07.08.2020: changed from “Termination of mice and rats with CO₂” to “Termination of mice and rats”.
New content + tables over chemicals. (Frøydis Kilmer)

6.4 09.03.2021: Appendix 1: Acute clinical conditions in mice and Appendix 2: Medicines that may be used
in the treatment of some conditions (by Katarzyna Joanna Zelewska) added. (Frøydis Kilmer)

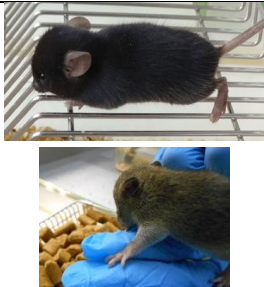


6.5 Revised and edited 28.06.2023 (Helene Tandberg and Katarzyna Zelewska)

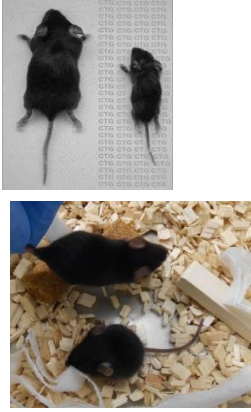
7.0 REFERENCES

7.1 Dyrevelferdsloven (Animal Welfare Act)

7.2 Forskrift for bruk av forsøksdyr (Regulation on the use of animals for research)



Appendix 1: Acute clinical conditions in mice




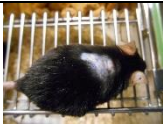
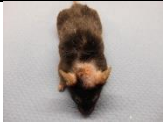
| Clinical symptoms | Description | Actions | Illustrations |
|---|---|-------------|---|
| Congenital abnormalities | | | |
| Hydrocephalus | The classical sign is an enlarged, domed head, ataxia and depression. | Termination |  |
| Malocclusion | A misalignment of the teeth or incorrect relation between the teeth of the upper and lower dental arches. This deformity means the animal has difficulty in eating and discomfort when chewing food. | Termination |  |
| Vaginal septum or imperforate vagina | The accumulation of secretions causes considerable distension of the vagina, cervix and womb and negatively affects reproduction. If the animal is mated, it can lead to difficulties during birth (dystocia) | Termination |  |




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| <p>Poorly developed pups</p> | <p>The young are weak and poorly developed, with phenotypic changes such as insufficient body weight, missing limbs etc.</p> | <p>Termination (check breeding plan against the FOTS project/user or supplier)</p> |  |
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Acute clinical conditions - MICE

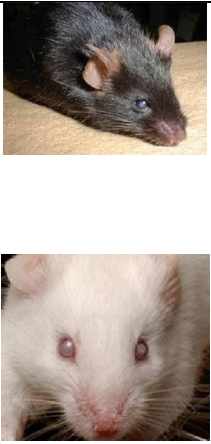

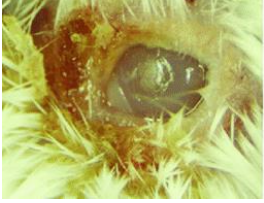
Always check the condition in accordance with FOTS

| Clinical symptoms | Description | Actions | Illustrations |
|---------------------------------------|---|---|---|
| <p>Weight loss/weight gain</p> | <p>< 5% weight loss</p> | <p>Check teeth, look for other clinical signs</p> | |
| | <p>5-10% weight loss</p> | <p>Check teeth, look for other clinical signs, place pellets and wet food on the bottom of the cage, weigh animals twice a week</p> | |
| | <p>10-20% weight loss</p> | <p>Check/correct the causes, use soft food or gel capsules, weigh the animals daily, inform the veterinary surgeon</p> |  |
| | <p>15- 20% <u>weight gain</u> and mobility difficulties</p> | <p>Immediate termination (NB! Check FOTS!)</p> |  |


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| <p>General behaviour (mobility, unusual/abnormal movements, arching the back, interaction in the cage)</p> | <p>Minor reduction in the level of activity, slow movements, slower to respond to stimuli, rather marked arching of the back, less interaction with other animals in the cage, some neglect of nest building</p> | <p>Increase the frequency of welfare monitoring, investigate possible causes and act accordingly, e.g. if arthritis, provide softer and deeper litter, extra warmth (paper) and consider the use of analgesics</p> |  |
| | <p>More obvious unwillingness to move (when the lid of the cage is removed), less awake than normal, repetitive movements/actions, limping, moderate back arching, piloerection, clear signs of isolation, aggressiveness</p> | <p>As above, frequent change of enrichment to help combat repetitive actions, e.g. introduce new toys, daily monitoring, give painkillers (consider oral administration), consider termination</p> |  |
| | <p>Little voluntary activity, isolated from other animals, large degree of piloerection, paresis, ataxia, worsening of earlier signs, seizures</p> | <p>Immediate termination</p> |  |
| <p>Ulcerative dermatitis (UD) (use a separate score sheet for UD if necessary)</p> | <p>Mild form: Alopecia (baldness), itching and red skin, lesions less than 1 cm in diameter</p> | <p>Consider chlorhexidine treatments on changed skin twice a day, clip nails on back feet every 10-14 days</p> |  |
| | <p>Moderate form: Intense itching, redness, scabs 1-2 cm</p> | <p>As above. If no improvement after treating twice, consider termination.</p> |  |
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| | <p>Severe form: Insistent itching, ulcerous lesions, open sores more than 2 cm, secondary bacterial infections</p> | <p>Immediate termination</p> |  |
| <p>Skin whitening or yellowing</p> | <p>Moderate form: slight whitening or yellowing of the feet, tail and eyes</p> | <p>Investigate possible causes, look for other clinical signs, if any, provide extra paper</p> | |
| | <p>Severe form: whitening or yellowing of the feet, tail and eyes</p> | <p>As above. Blood tests recommended to find the cause. Termination should be considered.</p> |  |
| <p>Prolapse (rectal, vaginal)</p> | <p>Mild form: diameter 1-2 mm, tissue is moist and pink</p> | <p>Consider use of painkillers, increase frequency of welfare monitoring, apply Vaseline locally, soft bedding, soft mat or gel capsules</p> | |
| | <p>Moderate form: diameter 3 -7 mm, vaginal/rectal. The affected area is swollen, the tissue is moist and pink, no</p> | <p>As above, analgesic treatment, clean the tissue with salt water, apply antibiotic Vaseline ointment.</p> |  |

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| | bleeding, impaired general condition | Termination should be considered | |
| | Severe form: more than 7 mm, vaginal/rectal area is swollen, the tissue is dark pink/red or black, dry and necrotic, bleeding, bad general condition | Immediate termination |   |
| Penis prolapse | Always severe form | Immediate termination |  |
| Exterior lumps/tumours | Small lumps | No treatment if they do not affect normal body functions. Increase the frequency of welfare monitoring |  |
| | Medium lumps (0,5-0,8mm), no ulceration or difficulty of movement | As above. Check for any other symptoms (lymph node enlargement). Welfare monitoring at least twice a week |  |
| | Large lumps (> 0,8 mm), mobility problems and/or ulcerous | Immediate termination |  |
| Eye problems | | |  |

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| | <p>Defects unlikely to affect animal health:</p> <ul style="list-style-type: none"> - Opaque cornea or cataract: white fleck on the eye (opaque cornea or opaque lens /cataract). The surface of the eye is smooth and there is no discharge - microphthalmia - anophthalmia - | <p>Must be distinguished from keratitis by means of a magnifying glass</p> <p>No treatment</p> |  |
| | <p><u>Conjunctivitis</u> - red/swollen eyelids with/without discharge</p> | <p>Consider the frequency of welfare monitoring, clip front and back nails once a week, apply antibiotic ointment, give painkillers. If corneal ulcers are present, termination is recommended.</p> <p>Medicines: sodium chloride for rinsing the eyes, Tobrex</p> |  |
| | <p><u>Keratitis</u> - the surface of the eye is irregular, with/without ulcers and with/without discharge</p> <p><u>Keratitis and conjunctivitis</u> - the surface of the eye is irregular, with/without ulcers, with discharge</p> | <p>Immediate termination</p> |  |

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| <p>Bite injuries</p> | <p>Less severe injuries: no large, bleeding wounds, usually on the tail and buttocks, the wounds appear dry</p> | <p>Check for genital injuries, increase the frequency of welfare monitoring, give extra enrichment or separate aggressive mice, give painkillers (Temgesic), sodium chloride 9 mg for cleaning, antibiotic ointment (Fucidin). Follow the rules for moving males during cage changing</p> |  |
| | <p>Serious injuries: torn skin, penile injuries, bleeding wounds, apathy</p> | <p>Immediate termination</p> |  |
| <p>Bloated abdomen</p> | <p>The abdomen is larger than normal in non-obese animals/animals not pregnant. The abdomen is tense with tousled fur</p> | <p>Check for pregnancy and obesity. Must distinguish between ascites, hydronephrosis and hydrometra/mucometra. If mobility is impaired: immediate termination</p> |  |
| <p>Dystocia</p> | <p>Pups are trapped in the birth canal, exposed uterus, arching</p> | <p>Immediate termination or Caesarean section and</p> | |

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| | of the back, weakness, heavy breathing | removal of the pups to another lactating female |  |
| Neurological disorders | Varying intensity: ataxia, head tilt, spinning when lifted by the tail, circling, cramps | Supportive care: place pellets and wet food on the bottom of the cage, extra paper. The maintenance of such mice should be evaluated in accordance with the experiment proposal or should be terminated | |

Appendiks 2: Termination of animals in SL

Termination types:

3W1 – NON-GM animals - NOT used for organ/tissue removal

For handling/immobilization training, research, surplus animals

3W2 - NON-GM animals used for organ/tissue removal

For animals used only for organ/tissue removal, without previous research (trials)

3GM1 - GM animals - NOT used for organ/tissue removal

For handling/immobilization training, research, surplus animals

3GM2 - GM animals - used for organ/tissue removal

Animals used only for organ/tissue removal, without previous research (trials)

3EW - Establishment of new GMO line. NON-GM animals (wild type offspring)

Here ONLY wild-type offspring from the establishment of GM lines are reported.

3EGM Establishment of new GMO line. GM animals.

Here ONLY GM-animals from the establishment of GM lines are reported.

3M - Maintenance of GM line. Both GM and wild-type offspring
Here GM animals and wild/type animals are reported

3F - Found dead

3S1 Sick animals in trials (regardless of genotype)

3S2 Sick animal not in trials (regardless of genotype)

Termination methods:

Cervical dislocation

Co2 chamber

Found dead

Halothane anesthesia followed by decapitation

Lethal i.p. injection Pentobarbital

Lethal i.p. injection ZRF cocktail

Stunning followed by decapitation

Degree of severity – we follow what is written in the FOTS application. This does not apply to animals found sick or dead or euthanized during/not during experiments, then the burden is higher than the original.

Non-recovery - applies to wild-type animals used in experiments where they are placed under general anaesthesia and euthanized while still under anaesthesia. In GM animals, the genetic modification itself is considered a procedure, therefore the GM animals can never be reported as "non-recovery"

Mild

Moderate

Severe

More information can be found in the SL "help" rubric.

Appendix 3: Medicines that may be used in the treatment of some conditions

| Medicines | Indications | Dosage | Contraindications |
|---------------------------------|---|---|--|
| Antiseptics | | | |
| Chlorhexidine | Disinfection of wounds, mucosal disinfection in the case of vaginal/rectal prolapse | According to need | Must not be used on joints and tendons. Must not come into contact with the brain, the meninges or perforated eardrums because chlorhexidine is neurotoxic |
| Chlorhexidine spirit | Skin disinfection before injections, puncture, sampling and surgery | Disinfect the skin surface and let the solution dry before the procedure | As above |
| Antibiotics | | | |
| Fucidin | Skin diseases: dermatitis, eczema | Apply 2-3 times a day for 5-7 days | Skin infections related to large, open wounds |
| Tobrex | Eye drops: watery eyes, conjunctivitis, keratitis | 1 drop twice a day for 5-7 days | None |
| Lubricating eye ointment | | | |
| Simplex | Sore or dry eyes | Apply a little ointment along the edge of the eye and eyelid or on the eye itself | None |
| Analgesics | | | |
| Xylocain | Local anaesthetic for painful surface, skin | Apply a little ointment on the affected area | |

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| | injuries, vaginal/rectal prolapse | | |
| Temgesic | Pain/discomfort according to scoring | Mice: 0,1 mg/kg/6-8t s.c. Rats: 0,05 mg/kg/6-8t s.c. | Severe respiratory insufficiency |

Appendix 4:

Extractor arm fume hood

