

Appendix 3: CoP for Rodents

Research that involves rodents uses both rats and mice. There are physiological and behavioural differences not only between mice and rats, but also between the different strains of mice and rats, as well as between individuals within a certain strain. Researchers must be aware of the specific traits (fluid and food intake, behavioural, physical) of the specific breeding lines/strains/individuals they are working with. Neurocognitive research conducted in the Netherlands uses fluid and food restriction in research involving mice and food restriction in research involving rats. Fully substantiated values for the minimum fluid volume per kg of body weight per day for rats are not available.

Guidelines for fluid or food restriction in rodents

General:

1. During the drafting process of the project description and the working protocol, in which fluid or food restriction is to take place, consultation is required between the responsible researcher and the Animal Welfare Body (IvD) regarding the design and the execution of the study, in particular concerning the extent, duration and manner of fluid and food restriction, the training of the animals and the determination of decision points and criteria for either interim adaptation, temporary suspension or premature termination of the protocol.
2. A project license application must scientifically substantiate the choice for a motivation method if fluid or food restriction is selected, as well as provide the results of a Synthesis of Evidence. It must also provide insight into the type of reward offered and method of dispensation.
3. The simultaneous application of fluid and food restriction is prohibited.
4. Pregnant animals may not be used for research in which fluid or food restriction is applied, unless explicitly permitted in the project license, following explicit scientific substantiation and careful consideration, and with an adapted degree of restriction.
5. The animals' living environment must be enriched in such a way that it encourages animals to make choices in their behaviour. This may mean offering an alternating variety of cage enrichment. In addition to cage enrichment, the animals must also be given a type of social enrichment.

Training:

6. Animals must have the opportunity to gradually adapt to the schedule of restriction and the fact that fluid/food can be obtained by conducting a type of task. The amount of time required for this will vary per species and per individual.
7. The individual weight of an animal must be determined prior to the protocol commencing. In the case of animals still in a phase of growth, a relevant control group/historical data pool must be used (growth curves of the suppliers are not suitable for this purpose due to the effect of the specific feed, the microbiological status of the animal facility, etc. on the growth curve). Correction may take place for the observation that animals on ad lib intake keep gaining weight after their growth period (fattening) by levelling off the control growth curve from adult age onward (approximately 2-3 months for mice and approximately 6 months for rats).

Restriction protocol:

8. The researcher responsible for the research must at all times apply a type of restriction that is the least taxing on the animals and which still allows the research objective to be achieved.
9. In protocols that use conditioning, the best type of reward and the best supply method must be ascertained based on the SoE or based on a pilot study. If necessary, a pilot study must be conducted beforehand.

10. The following absolute lower daily intake limits (i.e. this minimum must be provided every day) shall apply to animals (adult, not pregnant) subject to restriction:

	Rats	Mice
Fluid restriction ³⁴	No reliable values found	25 ml/kg/day
Food restriction	The amount of food that results in the animal not falling below the threshold of 85% of its body weight based on the growth curve of the relevant strain/ individual animal	The amount of food that results in the animal not falling below the threshold of 85% of its body weight based on the growth curve of the relevant strain/ individual animal

A higher supply must always be established, in consultation with the Animal Welfare Body, than is merely sufficient for the procedure, based on the strain used and dependent on the need of the animals. If the research uses the amounts such as are listed in the table above, then the necessity of such a choice must be substantiated in the project license application. The Animal Welfare Body is unable to recommend that an animal be given less than the amounts outlined in the table above.

- a. Animals that do not fulfil this established fluid intake requirement during the procedure, must be offered fluid that same day (in a familiar environment, such as the home cage or a cage specifically designed for that purpose), such that they still consume the required minimum daily amount of fluid.

- b. Animals that do not fulfil this established food intake requirement during the procedure, must be offered food that same day (in a familiar environment, such as the home cage or a cage specifically designed for that purpose), such that they still consume the required minimum daily amount of food.
11. Push days are prohibited: if the animal succeeds at fewer trials than expected, the animal may not be subjected to (temporary) more stringent restriction than is determined in the working protocol. Push days constitute an infringement of animal welfare due to the fact that fluid or food is kept from the animal for too long.
12. As soon as an animal ceases to carry out a task,³⁵ the session³⁶ must be halted. If, at that point, the animal has not yet consumed its daily minimum volume in fluid or food during the procedure, this must be provided until the animal has consumed this minimum amount. If the animal has consumed the minimum amount of fluid or food during the procedure, yet continues working, it must be given the opportunity to consume more fluid or food as a result.
13. Animals are rewarded using high-stimulant rewards, such as sucrose/saccharose pellets, sucrose/saccharose water or baby milk. The nature of what constitutes a highly stimulating reward may vary per specific animal or breed. The objective is to choose a reward that entails optimal fluid/food intake, which in some cases may be water.
14. The degree of restriction is tailored to the number of trials required, possibly in conjunction with the complexity/aversiveness of a task. The shorter in duration the task (possibly in relation to the complexity/aversiveness of a task), the less the animal may be restricted. No restriction is applied in cases of a complex/aversive task that need only be carried out a small number of times. *(please see decision tree on page 26)*
15. If the animal does not understand the task or is not inclined to perform the task, the complexity/aversiveness of the task must be reduced. In such cases, the fluid or food supply may not be lowered. In consultation with the person referred to in Article 14 of the Experiments on Animals Act (Wod, Article 25: designated veterinarian (2010/63/EU) and the Animal Welfare Body (IvD), it must be determined whether the fluid/food supply must be increased to counter (initial) deviating behaviour.
- Monitoring health and behaviour:*
16. Animals that are used in a fluid or food restriction protocol must be evaluated and assessed on a daily basis for potential clinical and behavioural changes. The findings must be recorded in the welfare log. Part of the assessment includes the monitoring/scoring of the animals' alertness, activity and physical condition. In addition, animals should be weighed regularly³⁷ and should be assessed for signs of dehydration.
17. All information about the animals, procedure and the monitoring findings and a description of individual monitoring deviations should be present or retrievable in the proximity of the animals.

18. The duration and the degree of the fluid or food restriction must be laid down in the working protocol, as well as any information on the training, key responsibilities, decision points and criteria for either the interim adaptation, suspension or premature termination of the protocol.
19. In order to monitor the growth of the animal, the animal's weight progression is compared with the standardised growth curves for the species, sex and breeding line before and during the procedure, taking into consideration the percentile in which the animal is situated during the acclimatisation period. Given that animals no longer grow in the case of ad lib intake but do grow fatter once they reach adult age, the growth curve may be levelled off at a maximum value after they have reached an adult age. If the animal exhibits a sharp drop or a sharply diverging growth curve and falls below the 85% threshold of its expected body weight, the animal must be (temporarily) removed from the procedure or its level of restriction must be limited. In the event of a sharp drop, in consultation with the Animal Welfare Body (IvD) and/or veterinarian, it must be determined how the animal should be treated (this may be laid down in the working protocol) and whether the procedure may be resumed. A sharp drop may also constitute a reason for a Humane Endpoint.
20. If the animal falls ill during the procedure or is recovering from an illness, or if the animal exhibits sharply deviating behaviour, a decision is made, in consultation with the Animal Welfare Body and/or veterinarian, whether the fluid or food regime should be adapted. An animal that is ill at the start of the experiment or procedure, or is recovering from an illness or operation, or exhibits deviating behaviour, may only be used in the research – and be subjected to restriction – once it has fully recovered. It is undesirable to allow animals that have been subjected to a restriction protocol to suddenly have unlimited access to fluid or food. Particularly with regard to fluid restriction this may result in excessive intake. An animal should be supplied with no more than the average voluntary fluid intake of the species (age, weight). Even in the case of short-term recovery periods and periods during which the animal is not participating in training, the researchers must ensure gradual transitions and a permanent, albeit light, restriction compared to voluntary intake. Restriction may be maintained during short-term interruptions (weekends) or the supply of fluid/food may be increased slightly (e.g. in the case of social housing where the supply is distributed per cage, to ensure that every animal consumes a sufficient amount). On both weekend days, an animal must at least receive the absolute minimum amount of fluid/food.
21. The parties responsible for the monitoring of the welfare of the animals must be demonstrably competent and qualified and must be able to recognise any deviations that indicate physical or behavioural changes.
22. If animals are required to undergo an operation during behavioural training, it is not recommended that animals be put on ad lib fluid and food intake and then be placed under restriction following their recovery. This is to avoid any major fluctuations in the animals' weight. The recovery period following an operation may



vary per species, type of operation and per individual. This will be monitored based on the clinical observation of the recovery.

Species-specific key areas of focus:

- Rodents live according to another circadian rhythm than humans. Given that their day and night rhythm is the reverse of that of humans, this must be taken into account in the research design. The restriction of animals during their active period will have a more significant physiological impact than any restriction of animals during their non-active period and may affect their welfare.³⁸ The behavioural task is carried out during their active period and any deviation from this should be substantiated in the project license application.
- Cage enrichment: an exercise wheel (or other movement stimulator) in conjunction with food restriction may result in caloric malnutrition and behavioural abnormalities (e.g stereotypy and disruption of the circadian rhythm) in rodents. In the event of sharp weight loss and behavioural abnormalities, the exercise wheel (or other movement stimulator) must be removed from the cage. (*Also see point 19*).

Appendix 5: Body Condition Score for rodents

Description of procedure:

Determining the Body Condition Score (BCS) in rodents is a non-invasive method to assess the health of the animals. BCS has a range of 1 (emaciated) to 5 (obese). An expected BCS of 1 or 2 requires intervention in the protocol (IACUC).

The BCS is determined by way of a visual and physical examination of the animal. The physical examination is carried out by gently holding the mouse by the base of tail and moving a finger across the sacroiliac joint. The figures below illustrate which findings correspond to which score. Scores must be documented per animal.

References

Ullman-Culleré, M.H., Foltz, C.J., (1999). Body Condition Scoring: a rapid and accurate method for assessing health status in mice. *Lab. Animal Science*; Vol 49 (3) 319-323.

Hickman, D.L., Swan, M., (2010). Use of a body condition score technique to assess health status in a rat model of polycystic kidney disease. *J Am Assoc Lab Anim Sci.* 49(2):155-9.

The Institutional Animal Care and Use Committee (IACUC);
Body Condition Scoring of mice

<http://www.iauc.ucsf.edu/Policies/awSPBodyCondScore.asp>

Figure 2: Body Condition Score for mice

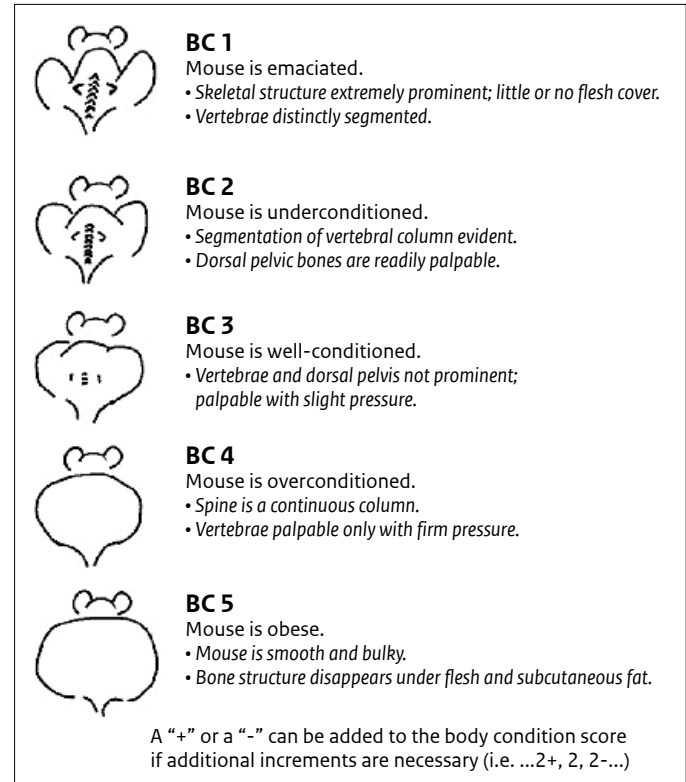


Figure 3: Body Condition Score for rats



BC 1

- Rat is emaciated.
- Segmentation of vertebral column prominent if not visible.
 - Little or no flesh cover over dorsal pelvis. Pins prominent if not visible.
 - Segmentation of caudal vertebrae prominent.



BC 2

- Rat is underconditioned.
- Segmentation of vertebral column prominent.
 - Thin flesh cover over dorsal pelvis, little subcutaneous fat. Pins easily palpable.
 - Thin flesh cover over caudal vertebrae, segmentation palpable with slight pressure.



BC 3

- Rat is well-conditioned.
- Segmentation of vertebral column easily palpable.
 - Moderate subcutaneous fat store over pelvis. Pins easily palpable with slight pressure.
 - Moderate fat store around tail base, caudal vertebrae may be palpable but not segmented.



BC 4

- Rat is overconditioned.
- Segmentation of vertebral column palpable with slight pressure.
 - Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis palpable with firm pressure.
 - Thick fat store over tail base, caudal vertebrae not palpable.



BC 5

- Rat is obese.
- Segmentation of vertebral column palpable with firm pressure; may be a continuous column.
 - Thick subcutaneous fat store over dorsal pelvis. Pins of pelvis not palpable with firm pressure.
 - Thick fat store over tail base, caudal vertebrae not palpable.