Preventing individual housing of laboratory animals

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1 Introduction

Dutch and European regulations [1, 2] state that laboratory animals must be housed in groups (two or more animals), unless the species is solitary by nature. In principle, all of the laboratory animal facilities at the Utrecht Science Park (USP) provide group housing for laboratory animals. Species that are solitary by nature, such as hamsters, are housed individually. In addition, there may be a variety of reasons, such as biological, welfare, experimental or practical reasons, that make it desirable or even necessary to temporarily or permanently house animals individually. In this regard, it is important to know that (long-term) individual housing has an effect on the behaviour and physiology of the animal, and thus not only affects the animal's welfare, but also the scientific results of the experiment. The regulations [2] state that in these situations the length of separation should be limited to the minimum necessary and contact through seeing, hearing, smelling and touching conspecifics should be maintained. Furthermore, the introduction or reintroduction of animals into existing groups should be carefully monitored to avoid problems resulting from incompatibility or disrupted social relationships.

2 Aim

This policy aims to:

- clarify when and under what conditions animals that live in groups by nature may be individually (singly) housed;
- set frameworks for determining when exceptions can be made to the obligation to attempt to re-introduce animals from individual back into group housing;
- give guidance for when to prevent or discontinue individual housing;
- align with national policies.

3 Who is this policy for?

This policy is binding for employees of UU and UMC Utrecht. External parties housing their animals in one of the UU or UMC Utrecht facilities are expected to comply with this policy. Exceptions can only be made in consultation with the Animal Welfare Body Utrecht (AWB).

4 General guidelines

- Researchers faced with the decision (planned or unplanned) to individually house animals used for an experiment must inform themselves of its expected effects on experimental results and must consider these effects in deciding whether or not to house animals individually.
- If the animals cannot be kept in group housing, they must be housed in such way that they can see, hear, smell and/or touch each other. Housing in IVC (individually ventilated cages) cages limits this contact to only being able to see animals in the neighbouring cage. Literature on the impact of IVC housing on the welfare of individually housed animals is currently insufficient.
- The researcher must also ensure additional enrichment of the environment (appropriate for the species), such as toys and/or extra attention from animal caretakers.

5 Situations and circumstances under which individual housing is permitted

The following laboratory animals may be housed individually, if the accompanying conditions (in italics) are met.

- Stabled horses in the Clinic for Equine Health.
  Horses in the pasture, except stallions, are kept in groups as far as possible, unless frequent group changes lead to ranking problems and additional stress. In stables, the horses must be able to see at least one other horse and have physical contact, either by using open-top boxes/stalls or a 'sniff hole' allowing them to touch noses. The Clinic for Equine Health is looking into expanding the possibility for free movement during the stable period, while also considering the safety of both animals and humans.
- Pregnant experimental animals from one day prior to parturition, or as much longer before that, as is necessary for the welfare of the mother and a proper birth process.

- All laboratory animals for which it can be assumed in advance that, based on strain, sex, or individual characteristics, they cannot (or can no longer) be housed socially, for example, due to aggression or microbial or immunological status.

  Additional conditions:

  - *The time and reason for individual housing must be recorded in the work protocol and procedures list.*
  - *Individual housing must be included in the CCD project application.*
  - *Based on consultation with the AWB, the designated veterinarian or clinic veterinarian, the researcher(s) will decide:*
    - *How long the animals are kept, also in relation to education/research.*
    - *Whether enough effort was made to prevent individual housing.*

- Sick animals or animals with a particular discomfort, in which case the purpose of individual housing is treatment and/or recuperation of the individual animal or to prevent contamination or stress to conspecifics. The fact that group housing may have a positive effect on the animal's recovery must be considered.

  Additional conditions:

  - *The AWB, the designated veterinarian or the clinic veterinarian must be consulted.*
  - *The reason for individual housing must be documented in the study folder.*
  - *Animals must not be kept in single housing longer than reasonably necessary.*

- Laboratory animals in experiment who must temporarily or permanently be housed individually in the interest of the study.

  Additional condition:

  - *Individual housing must be included in the CCD project application and work protocol.*

- Male laboratory animals used for breeding - in particular mice - who can no longer be housed in a group after mating.

  Additional conditions:

  - *The necessity must be mentioned beforehand in the study file.*
  - *Male mice to which this applies must be individually housed for as short a period as possible, not exceeding 20 weeks in total, with a maximum of 12 consecutive weeks.*
  - *Exceptions are only allowed based on consultation with the AWB.*

- Fish until genotyping results are obtained after fin-clipping or skin swab for up to five days.

  Additional conditions:

  - *The necessity must be mentioned beforehand in the study file.*
  - *The tanks in the genotyping setup have mutual water connection for the duration of individual housing.*

6 Discontinuing individual housing

If a situation occurs in which it seems possible to return individually housed animals to social housing (e.g., the last living animal in an experimental group, only weanling of either sex, victim of aggression), efforts must be made to end the individual housing. The following points are to be considered by the responsible researcher.

- The decision to stop individual housing in an experiment is made by the responsible researcher (or his substitute). He or she will ascertain if this aim is compatible with the research question and the proper execution of the experiment. He/she will also take into account critical factors such as sex, personality (e.g. in the case of dog or cat), microbial status, age and weight of the individual animal and/or its potential cage/housemate.
- A temporary or permanent separation can cause or exacerbate aggression in certain species, strains or sexes. This must be considered when deciding whether or not to return animals to group housing. If the decision is made to reunite the animals, their interactions with each other must be well monitored.
- If it is determined that the individual housing must be continued, the reason for doing so must be recorded. The maximum duration of this situation will be determined in consultation with the AWB.
- If problems occur after individual housing is discontinued which are reason to return the animal to individual housing (e.g. persistent aggression), then a second attempt to restore group housing does not need to be made. The animal will then be housed individually on a permanent basis. The maximum duration of this situation must be decided and documented in consultation with the AWB.
- Animals in experiments who are put in individual housing seven days or less before the planned end of the experiment need not be returned to group housing (regardless of the reason for single housing). Sometimes the period can be extended in consultation with the AWB.
- With large laboratory animals, situations may occur in which restoring group housing is possible, but it reduces the available space per animal to less than the legally required minimum. This is acceptable; group housing has priority in these kinds of situations. In all such cases the AWB must be consulted.

7 Prevention of individual housing

The following examples are to serve as inspiration in order to avoid/prevent individual housing of laboratory animals.

- *Reducing/preventing aggressive behaviour.* Various housing measures can help prevent aggressive behaviour between conspecifics.
- *Merging groups:* Animals in an experimental situation who are treated the same way, or whose treatment is not disturbed by cagemates, can be put together into new groups for the duration of the experiment. If this leads to problems in identification, a second form of marking can be used to distinguish animals with the same marks. This is only permitted if combining groups does not result in aggression between new group members.

- *Placement with buddies:* in both breeding and experiments, individual housing can be avoided by placing the animal in question with one or more animals, whether or not purchased in advance, that are not in the breeding or experiment.
- *Mixing species,* for example sheep and goats. These species must be known to get along with each other and get added value from the company of the others.
- *Placing a pup/young with breeding males,* for example with mice or rats. By inserting two consecutive breeding rounds with a breeding pair, the father can be weaned with the male offspring from the first litter.
- *Genotyping mice and rats before weaning age:* in this way, experimental groups/breeding stock of the right genotype can already be selected before weaning.
- *Strains with increased aggression:* let animals arrive at the animal facility at weaning age in order to form stable experimental groups.
- *Modifying the experimental tools or design:* Evolving knowledge and technological developments (e.g. more robust implants) may make individual housing during the experiment partially or wholly superfluous. It may also be possible to offer the animals a playtime in an enriched social environment or allow interim social housing.

Situation-specific measures and how to monitor their effects can be discussed with animal caretakers and animal technicians and/or the AWB.

8 Future prospects

This policy describes how UU and UMC Utrecht currently deal with the prevention of individual housing of laboratory animals. However, UU and UMC Utrecht are aware that this field is continually developing and are continually alert to developments that can improve the quality of research, as well as the 3Rs. This policy will therefore be evaluated regularly, at least every three years, and updated where necessary.

Currently we are working on implementation of the following subject(s) in relation to this policy:

- Exploring the possibilities of using chemically sterilised female buddy animals to prevent individual housing of male mice in experimental and/or breeding situations.

9 Relevant legislation and other sources

Annex III of Directive 2010/63/EU on the protection of animals used for scientific purposes contains rules for housing and caring of laboratory animals.

9.1 General

https://www.nc3rs.org.uk/3rs-resources/housing-and-husbandry

https://www.3hs-initiative.co.uk/

9.2 Rodents

Kappel S, Hawkins P, Mendl MT. (2017) **To Group or Not to Group? Good Practice for Housing Male Laboratory Mice.** Animals 7(12). pii: E88. doi: 10.3390/ani7120088.

Schipper L1,2, Harvey L3, van der Beek EM2,4, van Dijk G1 (2018) **Home alone: a systematic review and meta-analysis on the effects of individual housing on body weight, food intake and visceral fat mass in rodents.** Obes Rev. 19(5):614-637. doi: 10.1111/obr.12663. Epub 2018 Jan 15.

9.3 Dogs

Grigg EK[1,2], Nibblett BM[1], Robinson JQ[1], Smits JE[3] (2017) **Evaluating pair versus solitary housing in kennelled domestic dogs (*Canis familiaris*) using behaviour and hair cortisol: a pilot study.** Vet Rec Open. 2017 Jun 26;4(1):e000193. doi: 10.1136/vetreco-2016-000193. eCollection 2017.

9.4 Farm animals

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Costa JHC[1], von Keyserlingk MAG[1], Weary DM[2] (2016) **Invited review: Effects of group housing of dairy calves on behaviour, cognition, performance, and health.** J Dairy Sci. 99(4):2453-2467. doi: 10.3168/jds.2015-10144. Epub 2016 Feb 10.

9.5 Horses

Yarnell K[1], Hall C[2], Royle C[2], Walker SL[3] (2015) **Domesticated horses differ in their behavioural and physiological responses to isolated and group housing.** Physiol Behav. 143:51-7. doi: 10.1016/j.physbeh.2015.02.040. Epub 2015 Feb 25.

Hallmark Horse and Welfare (2018) Handbook Hallmark Horse and Welfare. http://keurmerkpaardenwelzijn.nl/wordpress/criteria-2/

10 References

1. Animal Experiments Act: https://wetten.overheid.nl/zoeken/zoekresultaat/rs/2,3,4/titel/dierproeven/titelf/1/tekstf/1/artnrb/0/d/20- 06-2019/dx/0
2. EU Directive 2010/63/EU, Appendix III: https://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2010:276:0033:0079:en:PDF

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