Project authorisation for breeding of genetically altered animals

Project authorisation is *required* for breeding genetically altered (GA) animals *with a likely or known harmful phenotype.*

A harmful phenotype is defined in the EU Directive and the Norwegian regulation as: an animal that is likely to experience, as a consequence of the genetic alteration, pain, distress, suffering or lasting harm equivalent to, or higher than that caused by the introduction of a needle in accordance with good veterinary practice[i].

Project authorisation is not required for breeding of GA animals *without a likely or known harmful phenotype*. The burden of proof to document that a GA line does not have a harmful phenotype is on the user.

The EU commission has provided formal criteria for assessing the phenotype of a GA lines. Mattilsynet also refers to these criteria[i].

At KPM-OUS, maintenance of an established GA line without project authorization requires documentation that the GA line is without a harmful phenotype by performing an initial Welfare Assessment. The initial welfare assessment must be completed according to the EU-commission´s "GA Rodent Welfare Assessment Scheme" (see attachment).

Terminology

Breeding of GA lines is divided in two phases; Creation and Maintenance.

Creation phase

Comprise the phase where a new GA line of animals is created, by genetic modification techniques. *Project authorisation for breeding of a GA line is always required* during the Creation phase. A GA-line will remain in the "creation phase" until an initial Welfare Assessment has been concluded.

Maintenance phase

The Creation phase of a GA line is superseded by the Maintenance phase when the new GA line is "established". The Maintenance phase of an "established" GA line requires a) stable transmission of the genetic alteration for at least 2 generations and b) completion of an formal Welfare Assessment.

Project authorisation requirements

If requirements a) and b) are completed and the initial animal welfare assessment conclude that the GA line has a known or likely harmful constitutional phenotype, Maintenance breeding of the GA line *requires project authorisation*.

If requirements a) and b) are completed and the initial animal welfare assessment conclude that the GA line *does not* have a likely harmful phenotype, Maintenance breeding of the GA line *does not require project authorisation*.

If requirement a) and b) are not completed, the GA line remains in the Creation phase, requiring continued project authorisation for breeding.

Conditional GA lines do not develop a harmful phenotype until intentionally induced. Breeding of conditional GA lines does not require project authorisation.

If a harmful phenotype is identified in a GA line, previously defined as being without a harmful phenotype, project authorization will be required.

Irrespective of project authorisation requirements for breeding, *project authorisation is required for all use of GA animals in experimental procedures*.

Simple ear marking

Simple ear marking, performed with the purpose of permanent animal identification, does not require project authorisation. This is also the case if the ear biopsy is used for genotyping in GA lines without a harmful phenotype. However, obtaining ear biopsies for the purpose of genotyping only (included repeated biopsies from an animal previously ear marked for ID purposes) do require project authorisation. If genotyping is required in a GA line without a harmful phenotype, users are strongly advised to acquire project authorisation such that repeated biopsies (misplaced biopsy, analytical error, other reasons) can be obtained without delay.

GA Rodent Welfare Assessment Scheme used for initial welfare assessment

Criteria	What to look for
Overall Appearance	Is the animal morphologically 'normal'? Are there any malformations or any other indicators that the phenotype has been affected? For example skeletal deformity or hydrocephalus.
Size, conformation and growth	Are there any deviations from expected size or growth curve?
Coat condition	Is there any piloerection, areas of fur loss, loss of whiskers, barbering? Is the skin / fur in good condition?
Behaviour – Posture, gait, activity and interactions with the environment	Do they exhibit the full repertoire of behaviours appropriate for the strain/species, including social interactions, grooming, walking, running, digging, climbing? Are these normal? Is the animal hunched or reluctant to move? Is movement impaired or is there any difficulty with orientation? Any signs of rigidity or tremors? Any abnormal activity levels? Prolonged inactivity could indicate chronic stress or depression (anhedonia) and/or sickness/pain, particularly if linked with a hunched posture and/or rough or unkempt coat. Unusual activity, such as hyperactivity, could indicate stereotypy or other behavioural abnormality.
Clinical signs	For example - nasal or ocular discharge, swollen or closed eyes; increased respiratory rate; dyspnoea; seizures/twitches/tremors; increased vocalisation with handling; overgrown teeth; presence of tumours, neurological or musculoskeletal abnormalities. Is metabolism impaired, for example, increased or decreased food or water intake, excessive urination? Consistency of faeces.
Relative size	Any unusual changes in size of the animals should be noted, and comparisons made within the litter. It may be helpful to generate a growth curve for the line.
Numbers	Where death occurs, it is important to maintain accurate records such that any pre- or post-weaning losses can be investigated. Where appropriate (e.g. higher than anticipated mortality rate), post mortem examinations should be carried out to help determine the cause of death. A review of fertility can also be helpful in assessment of whether or not the modification is having an effect e.g. conception rates; abortions; stillbirths.
Colour of pups (for neonate only)	Do any pups show evidence of abnormal skin colour (e.g. anaemia, poor circulation)
Activity of pups (for neonate only)	Any abnormal activity, e.g. reduced wriggling? Righting reflex intact?
Milk spot (for neonate only)	Do any pups fail to show presence of a milk spot? Any evidence of mis-mothering?
Litter	Litter sizes; litter homogeneity; development and growth of pups

The initial welfare assessment must adhere to above table and include:

1. Animals of representative age groups (neonatal, weanling, sexually mature and other relevant time points (e.g. age dependent phenotype)
2. A minimum of 7 males/7 females from more than one litter
3. Animals from a minimum of two breeding cycles from F2 onwards
4. Non-GA control animals

References:

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[i] https://www.mattilsynet.no/dyr_og_dyrehold/dyrevelferd/forsoksdyr/oremerking_av_smaagnagere_i_forsoksdyrvirksomheter.28964