National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes

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Working document on genetically altered animals

Brussels, 22-23 March 2012

The Commission established two Expert Working Groups (EWG) (to develop common format for statistical reporting and for the assessment of severity of procedures) to facilitate the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. All Members States and main stakeholder organisations were invited to nominate experts to participate in the work.

The EWG for the statistical reporting met several times in 2011. During their work it became apparent that some further understanding was needed as to how genetically altered animals are to be considered. To seek some clarity to some of the questions, the first meeting of the Severity Assessment EWG focused on the genetically altered animals in its meeting in December 2011.

The consensus reached for the understanding of how genetically altered animals are authorised and covered by the statistics is detailed in this document. The document is the result of the work of the different EWGs, discussions with the Member States as well as legal input from the Commission. It was endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU at their meeting of 22 - 23 March 2012, followed by the endorsement of the GA welfare assessment scheme (incorporated in the Annex) at their meeting of 11-12 July 2012.

The related articles of Directive 2010/63/EU

- **Article 1(2)** *" The elimination of pain, suffering, distress or lasting harm by the successful use of anaesthesia, analgesia or other methods shall not exclude the use of an animal in procedures from the scope of this Directive."*

- **Article 3(1)** *"'procedure' means any use, invasive or non-invasive, of an animal for experimental or other scientific purposes, with known or unknown outcome, or educational purposes, which may cause the animal a level of pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice.*

  This includes any course of action intended, or liable, to result in the birth or hatching of an animal *or* ***the creation and maintenance of a genetically modified animal line in any such condition,*** *but excludes the killing of animals solely for the use of their organs or tissues*;"

- **Article 4(3)** "*Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals*."

- **Article 17(1)** " *A procedure shall be deemed to end when no further observations are to be made for that procedure or, as regards new genetically modified animal lines, when the progeny are no longer observed or expected to experience pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle*."

General background

For the purposes of Directive, "genetically altered animals" include genetically modified (transgenic, knock-out and other forms of genetic alteration) and naturally occurring or induced mutant animals as per the definition in Article 3(1).

An animal with a harmful phenotype for the purposes of this Directive and in context of genetically altered animals is to be understood as an animal who 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.

Requirements for a project authorisation

Creation: a creation of a new genetically altered line requires a project authorisation until such time when the line is "established".

A new strain or line of genetically altered animals is considered to be "established" when transmission of the genetic alteration is stable, which will be a minimum of two generations, and an initial welfare assessment completed (see Annex).

Maintenance: the use of animals for the maintenance of colonies of genetically altered established lines, *with a likely harmful phenotype*, requires a project authorisation. However, this could be considered under multiple generic authorisation (Article 40.4).

The use of animals for the maintenance of colonies of genetically altered established lines *without* a likely harmful phenotype is not considered a procedure and thus does not require a project authorisation.

Genetically altered lines requiring a specific, intentional (non-accidental) intervention to induce gene expression (e.g. chemical induction, mating of Cre with appropriate Lox animals) can be considered as having a non-harmful phenotype until deliberate induction of gene expression. Therefore, their breeding does not require project authorisation.

Genetically altered lines which retain a risk of the development of a harmful phenotype (e.g. age onset of disease or tumours; risk of infection due to compromised immune system) regardless of the applied refinement (e.g. barrier conditions, culling at early age), in line with Article 1(2), their breeding requires project authorisation as the application of refinement does not eliminate the risk.

N.B. If welfare issues are later identified, these should be reviewed to consider whether the welfare problems may be attributed to the genetic alteration. If so, these should be re-classified as “harmful phenotypes” and brought back under project authorisation.

Use: use of genetically altered animals in a procedure requires a project authorisation. These animals may or may not exhibit a harmful phenotype.

Genetically altered animals in statistical reporting

- Genetically altered animals are reported either
    a) when used for the creation of a new line;
    b) when used for the maintenance of an established line bred under project authorisation *and* exhibited harmful phenotype or
    c) when used in other procedures (i.e. not for creation or for the maintenance of a line).
- **The creation of a new genetically altered line** requires a project authorisation until such time as the line is "established". All animals carrying the genetic alteration should be reported during the creation of a new line. In addition, those used for superovulation, vasectomy, embryo implantation should equally be reported (these may or may not be genetically altered themselves). Genetically normal animals (wild type offspring) produced as a result of creation of a new genetically altered line should not be reported.
- In category 'Purposes', the animals used for the *creation* of a new genetically altered line should be reported under 'basic research' or 'translational and applied research' in the *respective category the line is being created for*.

- **A new strain or line of genetically altered animals is considered to be "established"** when transmission of the genetic alteration is stable, which will be a minimum of two generations, and an initial welfare assessment has been completed (see Annex).

- The welfare assessment will determine if the newly created line is expected to have a likely *harmful phenotype* and the animals from this point onwards shall be reported under category ‘Maintenance of colonies of established genetically altered animals, not used in other procedures' – or in the other procedures they are being used for. If the welfare assessment concludes that the line is not expected to have a harmful phenotype, its *breeding* falls outside the scope of a procedure.

- **‘Maintenance of colonies of established genetically altered animals, not used in other procedures'** contains the animals required for the *maintenance* of colonies of genetically altered animals of established lines *with a likely harmful phenotype* and which *have exhibited* pain, suffering, distress or lasting harm as a consequence of the harmful genotype. The intended purpose for which the line is being maintained for is not recorded.

- **All genetically altered animals which are used in other procedures** (not for the creation or maintenance of a genetically altered line) should be reported under their respective purposes (the same way as any non-genetically altered animal). These animals may or may not exhibit harmful phenotype.

- Genetically altered animals, expressing harmful phenotype, and killed for their organs and tissue, should be reported under the respective primary purposes for which the organs/tissue were used.

Annex

Key Elements of a GA Rodent Welfare Assessment Scheme

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.

Additional considerations for assessment in Neonatal animals

CRITERIA	WHAT TO LOOK FOR
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