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Severity classification of genetically altered animals under the Animals (Scientific Procedures) Act 1986

Contents

Statistical reporting of genetically altered animals

All genetically altered animals (GAAs) bred under project licence authority should be counted in the annual Returns of Procedures.

From 2014 the actual severity of all regulated procedures must be reported as part of the annual Returns of Procedures. All procedures are counted at the end of the procedure (not at the start as has been the case up to 2013).

If they are not used on further protocols, GAAs should be counted when they are culled or die. This will enable the overall severity of breeding and maintenance to be assessed for both breeding stock and surplus stock.

Animals transferred for ‘continued use’ under a separate protocol should be counted at the end of that procedure and reported as used under that purpose (not under ‘maintenance and breeding’).

This will remove the difficulty of counting animals when their final fate is unknown.

General principles of severity assessment in genetically altered animals
Severity assessment of GAAs will depend on the use of each individual animal.

- If GAAs are used in further procedures (continued use from a breeding protocol to a final use protocol), the severity will reflect the entire procedure, including the experimental procedures and the phenotype (see also the general *Advisory notes on recording and reporting the actual severity of regulated procedures*).
- If animals are not used in further procedures, but solely on ‘breeding and maintenance’ protocols, the severity will reflect the harms caused by the phenotype and any regulated procedures applied as part of genotyping, phenotyping, etc.
- The prospective severity classification will reflect the expected phenotype taking into account the humane endpoints in the protocol, which might reduce what could potentially occur to an animal of a particular strain. The retrospective or actual severity assessment will reflect what actually happened to each individual, and this could be more or less severe than the prospective severity classification.

Counting offspring

While some categories of animal are protected prior to birth or hatching, for the purpose of statistical reporting the ‘regulated procedure’ involved in breeding and maintenance of GAAs is the birth, hatching or reaching free-feeding stage, as appropriate, of a genetically altered offspring if this has the potential to suffer. Further regulated procedures may include genotyping and phenotyping techniques.

Offspring are counted and severity assigned in the following cases.

- They are born (including by caesarean section when viable) and can be counted (i.e. ‘missing litters’ where no offspring were actually observed but it is assumed some were born, cannot be included).

- They are non-GAAs (for example, of heterozygous matings that result in wild-type offspring) but are invasively genotyped, phenotyped, etc.
- They have been born of genetically altered parents but have not been genotyped, and therefore may or may not be genetically altered. These should all be counted as GAAs unless an informed decision can be made based on a Mendelian distribution that enables a prediction from the breeding records of that colony. If there is doubt, the animals should be considered to be genetically altered.
- Larval forms reach free feeding stages for fish and amphibian (noting that there are a large number of losses in fish that are non-genotype related due to the natural breeding strategy).
- Avian or reptile eggs hatch.

Breeding stock (parents and any offspring not transferred to experimental protocols) severity should reflect the overall actual severity experienced for each individual, which will depend on the age or stage of phenotype progression when the animal is killed or dies.

Wild-type (WT) offspring of breeding stock are not usually included in statistical reporting, unless subjected to another regulated procedure, for example, phenotyping, etc.

Pairing and mating is not a regulated procedure.

Definition of WT strains: A WT strain occurs in an animal where the genotype has not been actively altered and a naturally occurring harmful mutation has not been deliberately maintained.

A natural harmful mutant: An animal that shows a deleterious phenotype, where there has been a spontaneous mutation that is harmful and the strain has been maintained (continues to be bred) for a scientific purpose.

A *genetically altered animal (GAA)* includes all genetically modified animals (transgenic, knock-out and other forms of genetic alteration) and mutations, whether naturally occurring or induced

As stated above, if animals are transferred from a breeding protocol to a final use protocol, the severity of the phenotype should be included in the overall severity assessment of the procedure, and they are returned under the purpose according to their final use (for example, applied studies).

Only if animals are never used in further procedures should they be returned under ‘maintenance of colonies’.

Severity assessment

The prospective severity classification of the breeding protocol will be the severity that has been determined on phenotyping of the lines/strains to be bred under that protocol. The retrospective, actual, severity assessment is based on the individual animal, **regardless** of the severity classification of the strain.

There are three possible causes of suffering on GAA breeding protocols:

- invasive genotyping (or phenotyping) methods;
- other regulated procedures authorised and applied on the breeding and maintenance protocol, such as administration of gene inducers;
- phenotype resulting from the genetic alteration.

Non-procedural harms

Common causes of suffering in normal animal colonies such as occasional fighting or occasional disease outbreaks should not be included, unless these were exacerbated by the genotype.

Harms such as, for example, low-level neonatal loss or fighting, which occur in wild type animals at comparable levels and are judged therefore not be caused by the genetic alteration, should not be included in the actual severity assessment (although genetically modified animals must still be assessed for the phenotype-related harms and counted). This requires the adverse effects of the ‘background’ strain to be known.

Techniques used primarily for identification are not regulated. If animals are genotyped using tissue generated as a by-product of an identification technique, then the severity of the biopsy should be disregarded. If the same technique is used to identify and genotype an animal simultaneously then this can be considered primarily for identification. If animals are not normally identified using this method then the technique is considered primarily for genotyping and is a regulated procedure.

Repeat biopsy for the purpose of genotyping must be regulated, as this will not be for the purpose of identification. This will require licence authority, including for animals that would otherwise be bred out with the Act as a non-harmful phenotype.

Procedural harms

In assessing the actual severity of a breeding procedure the 'procedural harms' include all such harms, intended and unintended. This includes phenotype effects in parents that result in harms to the offspring, for example, poor mothering.

To make inclusion of only procedural harms practicable the following rules should be followed.

- If the harm (for example, the rate of pre-weaning losses) is the same as in the 'background' strain, it should be disregarded.
- If the genetically altered strain shows harms above that in the background strain then *all of the harms should be included*, unless detailed information on the particular harms is readily available for the background strain. This avoids overcomplicating the assessment by requiring an attempted calculation of the genetically altered related component of suffering and provides the correct balance of transparency of harms with practicability for users. If adverse effects become apparent after some animals have already been assessed then changing of the severity assignment retrospectively is not required.
- If there is insufficient knowledge of the 'background' strain effect then all harms must be included.

Example of prospective and actual severity assessment for a genetically altered animal breeding protocol

Superoxide dismutase 1 (SOD-1) transgenic mice are commonly used in research on motor neuron disease. Mice are normal when born, develop onset of paralysis at approximately 80 days, show progressive weight loss, loss of righting reflex by 120 days and die shortly afterwards without intervention.

Because of the phenotype of the strain these animals must always be bred under project licence authority. Although the strain has a potentially very severe phenotype, the prospective severity classification of the protocol (what is expected) will depend on the endpoints specified in that protocol:
Prospective Classification

- endpoints that require all animals to be killed prior to 70 days of age are classified as mild;
- endpoints that require animals to be killed at the onset of paresis or weight loss are moderate;
- endpoints that require animals to be killed if they have lost 15 per cent of body weight but are not paralysed are moderate;
- endpoints that require animals to be killed at loss of righting reflex are classified as severe.

The actual severity assessment for offspring or breeding stock (what actually happened, regardless of the prospective severity assessment):

- animals killed prior to the onset of any clinical signs, whatever their age are classified as sub-threshold;
- animals killed when showing between 10 and 15 per cent weight loss
- and no other clinical signs are mild;
  animals showing early onset paresis or paralysis are moderate;
- weight loss of between 15 and 20 per cent with muscle wasting but without paralysis is classified as moderate;
- animals showing more than 15 per cent weight loss with paralysis are classified as severe;
  animals showing loss of righting reflex are classified as severe.

Severity classification

Mild

Prospective classification: Mild

Animals bred on a mild limit protocol will be one of the following.

- Unknown phenotype, but not expected to be harmful.
- Known phenotype that can be kept so that actual severity is sub-threshold or mild provided certain conditions are met. These conditions will be mandated through the project authorisation.
- Animals without a harmful phenotype that are to be genotyped using invasive methods, for example, tail tipping or other methods such as ear clipping, where this is not used primarily for identification. This will include both GAAs and non-GAAs to be genotyped within ‘breeding and maintenance’ protocols.

Retrospective: Actual severity assessed as sub-threshold

Animals can be considered sub-threshold if:

- they are born and no harmful phenotype actually manifested; or
- the phenotype was subtle and of no welfare significance; or
- because of the management conditions the phenotype caused no welfare concern to the animal.

Where the strain is known not to have a harmful phenotype and no unexpected noticeable adverse effects manifest, i.e. the animals *are overtly normal,* these animals can be classed as suffering sub-threshold severity (unless they have undergone a regulated procedure such as tissue biopsy for genotyping).

Where a harmful phenotype is backcrossed into another line, the harm should be considered to be transferred until there is evidence to the contrary.

A noticeable adverse effect is one that is observable to the naked eye during cageside monitoring, or evident on routine handling but does not require any specific tests to detect it.

However, if the line is known to have a disease that has onset from birth, then, regardless of appearance, a sub-threshold assessment cannot be given because the disease will already be present, for example, diabetes with onset of disease or pre-diabetic state from birth. These must be assigned a mild severity or higher.

Retrospective: Actual severity assessed as mild

A mild severity classification is appropriate when animals show a distinct phenotype but this does not:

- reduce lifespan compared with the background strain;
- prevent normal feeding and movement;
- result in systemic disease, but does lead to an observable difference in a parameter such as growth rate, size, anatomy or behaviour.

A mild severity classification might be given to:

- animals killed at the *onset* of a disease phenotype, provided it has not materially affected wellbeing;
- neonatal mice and rats lost due to the phenotype within the first five days after birth (note: this relates only to phenotype-related deaths, other procedure-related harms should be considered separately);
- invasive genotyping carried out using a method that causes only transient pain, even if the phenotype is not harmful;
- non-Schedule 1 methods of killing that are considered on the basis of evidence to be humane and rapid, where they have been performed correctly with no complication, for example, decapitation or cervical dislocation in animals above the weight specified in Schedule 1.

Moderate

Prospective: Moderate severity limit

Animals bred on moderate severity limit protocols will be expected to develop a phenotype that is sufficient to cause a disease comparable with the moderate severity description (see separate document *Advisory notes on recording and reporting the actual severity of regulated procedures*). Disease is not expected to be fatal, and animals will be killed before any disease becomes life-threatening.

Retrospective: Actual severity assessed as moderate

A severity classification of moderate could be given:

- for most harmful phenotypes that do not result in animals becoming severely ill, moribund or result in death;
- for most neoplasia if left beyond first detection but animals are killed within conventional limits (see Workman *et al*., *British Journal of Cancer* (2010) 102, pp 1555–1577);
- if animals show evidence of:
  - weight loss;
  - reduced weight gain compared with appropriate controls (for example, wild-type litter mates);
  - impairment of feeding sufficient to reduce body weight;
  - excessive body weight gain sufficient to induce disease;
  - significant compromise of normal movement; or

- clinical evidence of systemic illness within the limits of the moderate severity classification (see separate document *Advisory notes on recording and reporting the actual severity of regulated procedures*).

Severe

Prospective: Severe limit

Breeding protocols classed as severe will be unusual as this level of severity is often incompatible with breeding animals.

The most common examples will be breeding of heterozygous animals that will result in severe phenotypes in homozygous offspring, including:

- paralysis;
- seizures;
- fractures; or
- birth of living offspring with severe developmental abnormalities or expected pre-weaning death.

Retrospective: Actual severity assessed as severe

Breeding stock, or offspring, that are retained to a point where they show a disease phenotype in the severe category (see separate document *Advisory notes on retrospective recording and reporting of actual severity of regulated procedures*) will be classed as severe.

Any animals found dead on a breeding protocol, unless an informed decision (see separate document *Advisory notes on recording and reporting the actual severity of regulated procedures*) can be made that it is unlikely the death was preceded by severe suffering.

Offspring of rats and mice found dead or cannibalised after postnatal day five, or death of neonates in other species caused by aggressive maternal behaviour where offspring are well developed at birth (for example, pigs), where this is phenotype- and not husbandry-related. A husbandry-related cause of cannibalism would be facilities failure leading to stress in the parents, and therefore unrelated to that specific breeding programme.

Common examples of likely sub-threshold severity classification

- Categories of GAAs that are likely to be sub-threshold given the nature of the genetic alteration:
    - established heterozygotes carrying recessive alleles;
    - reporter gene expressing strains, where there is no adverse phenotype;
    - Cre expressing lines where they do not have other defects, for example, due to cross breeding to generate multi-gene manipulations.
- An animal of any strain that has not had a formal welfare assessment carried out and is bred under project licence authority if it does not show any noticeable adverse effects and has not been genotyped by a regulated method.
- Immunodeficient animals that do not during their lifetime suffer infections and remain otherwise healthy.
- Animals killed prior to the onset of any harmful phenotype in strains where the phenotype progresses over time but animals are overtly normal for the early part of their life.