DEUTSCHES ZENTRUM ZUM SCHUTZ VON VERSUCHSTIEREN

Bundesinstitut für Risikobewertung

Severity Assessment of genetically altered mice and rats - Version 2

Recommendation no. 002/2016 by the National Committee (TierSchG) dated 9 September 2016*

On 27 and 28 October 2014, the second workshop took place at the Federal Institute for Risk Assessment (BfR) on the topic of the severity classification of genetically altered mice and rats. The aim of the workshop was to revise criteria for the severity classification of genetically altered laboratory animals developed at the first workshop (June 2013).

The impulse for the workshops came from the new German Animal Welfare Act. When the new legislation came into force on 12 July 2013, breeding of genetically altered animal lines became subject to authorisation if individual animals of these lines might experience pain, suffering or lasting harm due to their genetic modifications. Hence, it is necessary to apply nationally standardised criteria for severity assessment in order to ensure uniform harm evaluation and to be able to access previously collected data when animals are transferred from one institution to another.

Representatives of the BfR, the German Society for Laboratory Animal Science, the Ludwig Maximilian University of Munich, the Technical University of Munich, the German Society of Pharmacology and Toxicology, the Max Delbrück Center for Molecular Medicine, and the Project Group of Authorising Agencies for Animal Experiments were involved in the workshop.

As a result of the second workshop, the document on “Definition of Criteria for Severity Assessment of Genetically Altered Laboratory Animals” was revised as well as the accompanying four forms for assessing genetically altered mice and rats.

The documents are presented as Version 2 of 21 July 2015. Revisions are highlighted in grey. With these documents, tools for the nationally uniform evaluation and documentation of the harm burden in genetically altered animals are available in connection with the compulsory authorisation provisions for the breeding of genetically altered animals.

The documents listed below follow:

1. Definition of Criteria for Severity Assessment of Genetically Altered Laboratory Animals - General section
2. Form: Assessment of newborn litter
3. Form: Assessment of litter on weaning
4. Form: Assessment of individual animal
5. Form: Final assessment of genetically altered lines
6. European working document on genetically altered animals (2013)

*This revised recommendation replaces the communication No. 029/2014 dated 25 July 2014. http://www.bfr.bund.de/cm/349/severity-assessment-of-genetically-altered-animals.pdf

Definition of criteria for severity assessment of genetically altered mice and rats

General section
Version 2 of 21 July 2015

With the coming into force of the new German Animal Welfare Act on 12 July 2013, the breeding of genetically altered animal lines was made subject to authorisation if individual animals of these lines might experience pain, suffering or harm as a consequence of genetic modification.[1]

As existing genetically altered lines are often bred not only in one but in several institutions at the same time, it is necessary to apply standardised criteria for severity assessments in order to ensure uniform harm evaluations and to be able to access previously collected data when animals are transferred from one institution to another.

This document is a result of the second workshop on severity classification of genetically altered laboratory animals at the Federal Institute for Risk Assessment (BfR) in October 2014; it is based both on the recommendations of the first workshop on this topic in June 2013 and of the Expert Working Group of the European Commission dated January 2013[2].
The document comprises a general section and four forms for the welfare assessment of genetically modified animals at different points in time. The European "Working document on genetically altered animals" of the competent national authorities for transposition of Directive 2010/63/EU of January 2013 is attached as an Annex with explanations and additions of the workshop participants.

In accordance with section 11 of the German Animal Welfare Act breeding and keeping of vertebrate animals or cephalopods are subject to authorisation by the competent authorities, irrespective of the authorisation requirement mentioned above. Part 1 “Keeping of vertebrate animals or cephalopods used in procedures or other scientific purposes“ of the German Laboratory Animal Welfare Regulation (Tierschutz-Versuchstierverordnung) specifies the requirements for the establishments concerning care and accommodation of animals.

Basic principle: In order to permit severity assessment of a genetically altered animal line, animals should be used that the user already has in the breeding programme until procedures are carried out. Animals shall not be additionally bred or killed for the purpose of severity assessment. This means that for the purpose of assessment only animals shall be used that are being bred or killed anyway. As far as possible animals from the corresponding background strain shall be used to control for identified changes.
Only animals with the desired genotype shall be used for assessment purposes.

[1] Third Act on Amendment of the German Animal Welfare Act of 4 July 2013 (Federal Law Gazette I No. 36, 12 July 2013, p. 2182).
[2] European Commission, Environment Directorate-General: National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes - Corrigendum of 24 January 2013 - Working document on genetically modified animals. Brussels, 23-24 January 2013.
http://ec.europa.eu/environment/chemicals/lab_animals/pdf/corrigendum.pdf

1. General procedure

a. Assessment/Observation period: from birth until either removal from breeding or beginning of experimental use. In the case of lines for which an assessment already exists for a defined period of time but which are kept by a new user beyond this period, the new user must conduct further observations. This applies irrespective of whether harm was previously detected or not.

b. Number of animals to be assessed: per line, at least 14 individuals of both sexes (7 male and 7 female animals) from different litters; unless a phenotype can only occur in one sex; in this case, at least 7 animals of the relevant sex shall be assessed.

c. For existing and adequately characterised lines that are introduced to an institution for the first time, previously collected data (e.g. data sheet of the breeders) shall be used to complete the assessment.

2. Assessment criteria

a) General provisions
see forms for the "Assessment of newborn litter", for "Assessment of litter on weaning" and for "Assessment of individual animal"

- The first assessments shall be undertaken in neonatal animals - "Assessment of newborn litter" - and on the day of weaning ("Assessment of litter on weaning").
- Further assessments are carried out at an age of two months and afterwards at intervals of three months ("Assessment of individual animal"). If conspicuous changes are observed that indicate a harmful phenotype at one of the assessment points or beyond, the intervals should be diminished accordingly.

b) Assessment of embryonal lethality
see also the form “Assessment of newborn litter”

- Section 14 of the German Laboratory Animal Welfare Regulation states explicitly that requirements for the authorisation of animal experiments shall also apply to procedures, in which foetal forms of mammals as from the last third of their normal development are used or intended to be used.
- An increased embryonal lethality occurring as a result of a genetic modification during the last third of gravity can lead to burdens for the foetuses and especially for the dams. Therefore, signs indicating embryonal lethality are to be taken into consideration while assessing newborn litter.

c) Assessment of newly created or imported lines (after the coming into effect of the new German Animal Welfare Act):

- All newly generated genetically altered animals modified by embryonal manipulation (transgenesis, homologous recombination, enzyme-mediated mutation, transduction etc.), by irradiation or by treatment with mutagenic substances are assessed using the forms specified under 2.a.
- In the case of generation of new lines by cross-breeding two lines with no harmful phenotype, an assessment must only be carried out using the aforementioned forms if the crossbred offspring is expected to show a harmful phenotype.
- Crossbred offspring of genetically altered lines where a harmful phenotype is expected are assessed using the forms specified under 2.a.
- All newly imported, genetically altered lines which have not been adequately characterised are assessed using the forms specified under 2.a.

d) Assessment of lines with inducible genetic modifications, "reporter genes" or other genetic modifications

- Lines in which the genetic modification of the phenotype only occurs due to treatment with inductors (e.g. tamoxifen, tetracycline etc.) are considered not to have a harmful phenotype until the time of induction and are not subject to authorization.
- Lines in which a genetically based phenotype is suppressed by treatment with substances added to the feed or drinking water are also considered to have no harmful phenotype and are therefore not subject to authorisation until the point the substances are discontinued in an authorised animal experiment.
- The presence of reporter genes in the genome and molecules arising from these genes do not result in a harmful phenotype per se. Therefore, breeding of lines into which only reporter genes were introduced is not subject to authorisation.
- Immunodeficient lines must be kept under conditions that reliably prevent infectious diseases. These conditions include appropriate housing systems (isolators, IVCs, ventilated cabinets, barriers), organisational measures (hygiene monitoring, disinfection programmes, rederivation by embryo transfer, sanitation programs) and personnel measures (education and training of staff responsible for the management of animal facilities, taking care of animals and carrying out animal experiments). Therefore, breeding of immunodeficient lines does not have to be authorised.[3]
- Wild-type animals from common inbred or outbred lines or wild-type animals without standardised genetic background and recombinant inbred lines or comparable variants are not considered to be genetically modified animals. They are not subject to authorisation and do not need to be assessed using the aforementioned forms.
- Lines that are not expected to have a harmful phenotype due to the nature of their genetic modification (e.g. Cre strains, Flox strains) are not subject to authorisation and do not need to be assessed using the aforementioned forms.
- Lines which develop harmful tumours due to a genetic modification categorically require authorisation (also in cases in which the tumours only occur from a certain age).
- Spontaneous mutations that - when they occur - are to be specifically bred further and are expected to show a harmful phenotype are subject to authorisation.

e. Assessment of lines that were part of the managed stock before the new Animal Welfare Act came into effect:

- As some of the existing animal lines have already been bred and used in the various institutions for a number of years, sufficient information concerning their behaviour and development as well as potential severity classification is generally available. For this reason, only the existing lines for which no adequate information on harm can be presented need to be assessed using the aforementioned forms.
  However, suitable documentation must be provided for lines with no harm in the future in order to permit verification of the classification "no harm".

[3] This assessment is currently being discussed (as of July 2015), despite consensus opinion of the workshop participants and requires further clarification.

3. Final assessment

The final severity assessment of a genetically modified animal line is made by the project manager in consultation with the responsible animal welfare officer. Where, in addition to the general criteria specified in this document further examinations are carried out to assess the severity of a genetically altered line and special findings are available, these shall be included in the final assessment.
The final assessment is summarised in the form "Final assessment" and shall be submitted to the authorities.

4. Designation of the genetically altered animal lines in filed applications

In order to avoid infringements of patent or copyright, an institution only has to specify an in-house designation for the applied-for line when filing an application for a new, genetically altered animal line. The applicant must notify the authority of the exact designation of the new line in accordance with international nomenclature immediately following the scientific publication of the line.

Form
Version 2 of 21 July 2015

„Assessment of newborn litter “*

*at the latest during the first cage change

Location (Institute and room): _____
_____
_____

Husbandry system (e.g. IVC, conventional cage, filter top, isolator etc.; hygiene status where applicable):
_____

Owner: _____
_____

Origin (Name of breeder, external institution etc.):
_____

Line (international designation):
Only needs to be specified after publication of the line
_____
_____

Current particularities (e.g.: noise due to construction site, sanitation activities, relocation of rooms etc.):
_____
_____

Line (internal designation): _____

Designation of the altered gene(s): _____
_____

Genetic background of the line: _____
_____

Expected phenotype (brief description): _____
_____

Dam no.: _____ Sire no.: _____ Litter Born on: _____ Generation: _____

Number born: _____ Date of assessment: _____

Signs indicating embryonal lethality: _____
_____

Colour of pups	□ normal	□ Abnormalities (please specify, e.g. pale)	
Activity of pups	□ normal	□ Abnormalities (please specify, e.g. conspicuous restlessness)	
Size, development of pups	□ homogeneous	□ not homogeneous	Weight □ normal □ reduced □ increased
Milk spot	□ present	□ not present	
Care by dam	□ normal	□ Abnormalities (please specify, e.g. neglect, cannibalism)	
Other conspicuous signs:			

Name of assessor: _____

Form
Version 2 of 21 July 2015

„Assessment of litter on weaning “

Location (Institute and room): _____
_____
_____

Husbandry system (e.g. IVC, conventional cage, filter top, isolator etc.; hygiene status where applicable):
_____

Owner: _____
_____

Origin (Name of breeder, external institution etc.):
_____

Line (international designation):
Only needs to be specified after publication of the line!
_____
_____

Current particularities (e.g.: noise due to construction site, sanitation activities, relocation of rooms etc.):
_____
_____

Line (internal designation): _____

Designation of the altered gene(s): _____
_____

Genetic background of the line: _____
_____

Expected phenotype (brief description): _____
_____

Dam no.: _____ Sire no.: _____ Litter Born on: _____ Generation: _____

Number born: _____ Number weaned: _____ Difference born/weaned: _____

Identification Number								
Weaning date								
Sex								
Body weight								
Conspicuous signs(1) Please use letters (see footnote)!								
Identification Number								
Weaning date								
Sex								
Body weight								
Conspicuous signs(1) Please use letters (see footnote)!								

Conspicuous signs prior to weaning	Date:			
	Conspicuous signs[1]: Please use letters (see footnote)!			

Conspicuous signs[1]:

a = No conspicuous signs
b = Areas of fur loss
c = Runt
d = Bite wounds
e = Microphthalmia
f = Abnormal teeth
g = Hydrocephalus
h = Other (please state)

Date: _____ Name of assessor: _____

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Form
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"Assessment of individual animal"
First assessment at the age of 2 months, then every 3 months*

*in the event of conspicuous signs, the examination intervals are to be reduced

Line (internal designation): _____ Line (international designation):

Only needs to be specified after publication of the line!

_____ _____

Owner: _____ Husbandry system: _____

Animal no.: _____ from litter on: _____ Generation: _____ Sex: _____ Genotyp: _____

In the case of conspicuous signs, please enter the relevant letter!
(see code at bottom of table)
Multiple conspicuous signs may be selected!

Date								
Name of assessor								
	normal	conspicuous	normal	conspicuous	normal	conspicuous	normal	conspicuous
Nutritional status[1]								
Posture[2]								
Behaviour and motor function[3]								
Coat and orifices[4]								
Reaction to handling[5]								
Other[6]								
Weight (g)								
Datum								
Name of assessor								
	normal	conspicuous	normal	conspicuous	normal	conspicuous	normal	conspicuous
Nutritional status[1]								
Posture[2]								
Behaviour and motor function[3]								
Coat and orifices[4]								
Reaction to handling[5]								
Other[6]								
Weight (g)								

[1] Nutritional status:
a = Emaciated
b = Overweight
c = Dehydrated

[2] Posture:
a = Crooked
b = Cowering

[3] Behaviour and motor function:
a = Segregation
b = Apathetic
c = Stereotypes
d = reduced Motion
e = Paralysis
f = Spasms

[4] Coat:
a = Ruffled
b = Dirty

Orifices:
c = Red tears
d = Diarrhoea/Discharge

[5] Reaction to handling:
a = Aggressive
b = Timid
c = Apathetic

[6] Other:
a = Tumors
b = Skin inflammation
c = Injuries
d = Cannibalism
e = Vocalisations
f = Rectal prolapse
g = Other (please specify)

Date of death and particularities during autopsy:

_____
_____
_____
_____

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Form
Version 2 of 21 July 2015

Final assessment of genetically altered lines

Institution and address: _____

Street: _____ Postcode: _____ Town: _____

Assessed line (international designation):
Only needs to be specified after publication of the line!
_____

Assessed line (internal designation):
_____

Description of genetic alteration(s) leading to harm if not yet described in databases:
_____
_____

Husbandry system of assessed animals: _____
_____

Gene loci and considered genotypes:
_____
_____

Assessed animals

Totel number: _____ of which female: _____ and male: _____
Average age of the animals at the time of final assessment: _____ ± stand. dev.: _____
Average no. of assessments per animal: _____ ± stand. dev.: _____

Conspicuous signs in terms of:	Occurred:	Number of animals affected	Conspicuous signs in terms of:	Occurred:	Number of animals affected
Nutrional status	Yes ☐ No ☐		Tumor	Yes ☐ No ☐	
Posture	Yes ☐ No ☐		Skin changes	Yes ☐ No ☐	
Reaction to handling	Yes ☐ No ☐		Injuries	Yes ☐ No ☐	
Coat/Orifices	Yes ☐ No ☐		Cannibalism	Yes ☐ No ☐	
Behaviour	Yes ☐ No ☐		Rectal prolapse	Yes ☐ No ☐	
Motor function	Yes ☐ No ☐				
Other conspicuous signs: _____					
Multiple conspicuous signs in individual animals: (Explanations on this point in the final assessment)	Yes ☐ No ☐				

Female animals

Mean litter size per female animal: _____ ± stand. dev.: _____

Average rearing losses: _____
(difference born – weaned ± stand. dev.)

Signs indicate harm burdens resulting from further investigations

_____
_____

Final assessment:

(if necessary, please use extra sheet) – evtl. streichen, wenn im Dokument Zeilen hinzugenommen werden können

The harm burdens are classified as none □ mild □ moderate □ severe □.

Reasons:
(comprehensible description of the characteristics of the harm, conspicuous signs need to be described and assessed)

_____
_____

The described harm occurred from an age of _____ weeks with a frequency of _____% of the examined animals.

In the event of harm, it is recommended that offspring of this line will be killed at an age of ____weeks if this is not contrary to the purpose of the project. The following refinement measures are recommended to reduce the potential harm:

_____
_____

Names of Project manager and animal welfare officer: _____
_____

Place: _____ Date: _____
Noted: _____
(Project manager and animal welfare officer)

National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes
Corrigendum of 24 January 2013
Working document on genetically altered animals
Brussels, 23-24 January 2013

English version with explanations and recommendations of the workshop "Documentation and publication of the severity classification of genetically modified laboratory animals" at the Federal Institute for Risk Assessment Berlin, 20-21 June 2013

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. A corrigendum to the Annex was endorsed on 24 January 2013.

Disclaimer:
The following is intended as guidance to assist the Member States and others affected by this Directive to arrive at a common understanding of the provisions contained in the Directive. All comments should be considered within the context of Directive 2010/63/EU on the protection of animals used for scientific purposes.

Only the Court of Justice of the European Union is entitled to interpret EU law with legally binding authority.

On 20 and 21 June 2013, the Federal Institute for Risk Assessment (BfR) staged a workshop on the "Documentation and publication of the severity classification for mouse lines". The "Working document on genetically altered animals" was translated into German, and explanations and recommendations were added. These additions, resulting from the BfR workshop, appear shaded in the working document, so that they can be easily identified.

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 theuse 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.

There is a consensus among participants at the BfR workshop that the definition of genetically modified animals refers only to individual gene loci and not the whole genome of animals. Consequently, wild-type animals from common inbred or outbred strains or wild-type animals without standardised genetic background and recombinant inbred strains or comparable variants do not constitute genetically altered animals and are therefore not subject to authorisation for breeds under the animal welfare legislation.

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".

There is a consensus among the participants of the BfR workshop that the creation of genetically altered animals by embryonal manipulation (transgenesis, homologous recombination, enzyme-mediated mutation, transduction etc.) or by irradiation or treatment with mutagenic substances is subject to authorisation. New genetically modified lines can also be obtained through cross-breeding (e.g. to create a new combination of genetic modifications or back-crossing of genetic modifications to create new genetic backgrounds). In case of new lines created by cross-breeding, the breed is only subject to authorisation if harm is expected for the cross-bred offspring. For example, the back-crossing of recessive knock-out alleles to a new genetic background is not subject to authorisation, when no homozygous genotypes are generated. A further example is the production of inducible expression systems by means of cross-breeding; this is also not subject to authorisation as long as no induction takes place.

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).

There is a consensus among the participants of the BfR workshop that, in the case of cross-breeding, a new genetically modified strain is considered to be established if animals with the desired genotype occur and their basal severity assessment is completed.

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.

There is a consensus among the participants of the BfR workshop that there is no authorisation obligation for strains in which either no harmful phenotype is found during a severity assessment or in which no harmful phenotype is expected as a result of the type of genetic modification (e.g. reporter strains, Cre strains, Flox strains).

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.

There is a consensus among the participants of the BfR workshop that breeding of Cre and Lox strains is not subject to authorisation per se, but that the cross-breeding of these strains to create a conditional variant may require authorisation. Conditional strains in which the common Cre gene is used do not constitute inducible systems. The breeding of Cre / Lox systems in which the offspring may exhibit a harmful phenotype (as, for example, with conditional knock-out animals) is subject to authorisation under the animal welfare legislation. In contrast, conditional systems using inducible Cre forms like CreER or CrePR concerns inducible systems, the breeding of which is not subject to authorisation as long as no induction takes place. Moreover, there is a consensus that inducible strains in which the system is deactivated by administering the suppressor tetracycline via drinking water and activated via withdrawal of the suppressor (so-called "tet-off systems") should also not

require authorisation under the animal welfare legislation, as long as the animals reliably receive the suppressor.

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.

The participants of the BfR workshop are of the opinion that the breeding of exclusively immunodeficient strains does not require an authorisation under the animal welfare laws if - in keeping with the refinement principle - harm of the animals can be ruled out by protecting them from pathogens ensuring appropriate housing conditions. They evaluate that the hygiene condition is a critical point during the severity assessment of immunodeficient animals and adequate hygiene conditions shall be reliably assured in the laboratory animal facilities. They therefore propose to request an authorisation for immunodeficient strains depending on the hygiene condition of the immunodeficient breeds.[4]
The participants of the BfR workshop support the view that strains that develop harmful tumors due to a genetic modification should be categorically subject to authorisation (also in cases in which the tumours only occur from a certain age). These kinds of tumor models should be replaced by inducible models.

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 reclassified 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.

The participants of the BfR workshop point to the fact that notifications of animal experiments are also possible in Germany under certain circumstances in accordance with Section 8a of the Third Act on Amendment of the German Animal Welfare Act of 4 July 2013 (Federal Gazette I No. 36, 12 July 2013, p. 2182).

[4] This assessment is currently being discussed (as of July 2015), despite consensus opinion of the workshop participants and requires further clarification.

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.

  The participants of the BfR workshop are of the opinion that reporting of animal numbers is only necessary for the creation of genetically altered animals by means of cross-breeding if the cross-breed is subject to authorisation (on this issue, see "Requirements for a project authorisation – "Creation") and the genotypes in question are genotypes that can lead to a harmful phenotype.

- 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

Include animals of representative age groups
• soon after birth, around weaning and again following sexual maturity*)
• a minimum of 7 males and 7 females sampled from more than one litter
• data from a minimum of two breeding cycles (from F2 onwards)
• comparisons made wherever possible with similar non GA animals.

*) and at additional time points as considered appropriate by a prospective review of the potential impact of the gene alteration e.g. where there is an age dependent onset of disease

There is a consensus among the participants of the BfR workshop that animals with the desired genotype should be used for the severity 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.

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

The participants of the BfR workshop are of the opinion that the criteria for severity assessment listed in the Annex to the working document are suitable and should be implemented. Proposals were drawn up for suitable forms to document line-specific harm.