

Severity assessment of genetically altered fish (bony fish, teleost fish)

Recommendation no. 001/2015 by the National Committee (TierSchG) dated 06.08.2015

On 11 and 21 June 2015, a workshop took place at the Federal Institute for Risk Assessment (BfR) on the topic of the severity classification of genetically altered fish.

The impulse for the workshop derived from the amendment of the German Animal Welfare Act (TierSchG; BGBl. I S. 1308). Since July 2013, breeding genetically altered animals is subject to authorisation if the progeny may experience pain, suffering or harm due to the genetic modification.

New generated, but also imported, genetically altered animal lines, have to be judged, if they are impaired or not. With respect to the fact that existing genetically altered lines are often breed not only at one institution, but at several institutions, and, respectively, transferred from one federal state to the other, it is urgent to use the same criteria for the severity assessment across Germany. A uniform severity assessment allows referring to already existing data when exchanging animals between institutions.

Aim of this workshop was to develop criteria for the severity classification of genetically altered fish, particularly of bony fish (teleost fish) according to the severity assessment of genetically altered animals (mice, rats; see communication no. 029/2014 of the BfR).

Participant of the workshop were representatives of the BfR, the German Society for Laboratory Science (GV-SOLAS), the Max-Planck-Institute for Neurobiology in Martinsried, the Albert-Ludwigs-University Freiburg, the Technical University of Munich, the Julius-Maximilians-University Würzburg, the Ruprecht-Karls-University Heidelberg, the Karlsruher Institute of Technology (KIT), the Friedrich-Loeffler-Institute (FLI) in Greifswald-Insel Riems, the Animal Welfare Working Group of the Federal State Working Group for Consumer Protection and the Project Group of the Competent Authorities.

During the workshop General information and 3 forms were elaborated for the severity assessment of genetically altered fish (bony fish, teleost fish).

With these documents, tools are now available for a nationwide uniform assessment and documentation of the impairment of genetically altered fish. The documents are presented as version 1.2 dated 06.08.2015. They present the current state of scientific knowledge.

Annex

- Form 1 - Assessment of teleost fish - Larvae at the time of independent feeding
- Form 2 - Assessment of teleost fish - Adult, sexually mature animals
- Form 3 - FINAL ASSESSMENT Teleost fish

1 General information for the severity assessment of genetically altered fish (teleost fish)

1.1 Legal background

With the amendment of the Animal Welfare Act (TierSchG; German Federal Gazette (BGBl. I, p. 1308 dated 4/7/2013), breeding genetically altered animals has become a procedure subject to regulatory approval, if it is to be expected that the offspring may suffer pain, suffering or harm due to the genetic modification, (§ 7 Para. 2 in conjunction with § 7a Para. 5 TierSchG). According to § 14 No. 1 of the Regulation on the Welfare of Animals Used in Experiments (TierSchVersV), these regulations are also applicable to independently feeding larval forms of vertebrae.

The consequences of this are as follows:

1. Once a genetically altered fish line has been established, it must be determined whether the offspring of this line is expected to suffer pain, suffering or harm. In order to make this decision, animals are assessed that will be used in further breeding (hereinafter: *severity assessment*).
2. A severity assessment must also be conducted for already established lines, unless there is scientific evidence (for example through publications) that no pain, suffering or harm is to be expected in the offspring.
3. A severity assessment of new genetically altered animal lines can be omitted if the possibility of the offspring suffering pain, suffering or harm resulting from the genetic modification can be excluded based on scientific evidence.

Under the heading “General background”, the European working document on genetically altered animals from January 2013 defines “genetically altered animals” as genetically modified (transgenic, knock-out and other forms of genetic alteration) as well as naturally occurring or induced mutant animals as per the definition in Article 3 (1) of the Directive 2010/63/EU. This definition differs from the definition of genetically modified organisms in the German Genetic Engineering Act (GenTG). In § 3 No. 3 GenTG, a genetically modified organism is defined as an organism “whose genetic material has been changed in a way that does not occur under natural conditions, i.e. through crossing and natural recombination”. Since the German Animal Welfare Act and the Genetic Engineering Act pursue different protection goals, their definitions of genetically altered animals are not congruent. The two legal sectors must be strictly separated. Regarding the relevant questions of the severity assessment, only the regulations of the Animal Welfare Law (both national and EU) shall be applied.

A genetically altered fish line is generally deemed to be established starting with the F2 generation. (Note: G0 = typically genetically mosaic transgenic fish growing from injected one-cell stages or, in the case of chemically or radiation-induced mutagenesis, animals mutagenized in the germline; F1 = first filial generation in which individual animals carry genetic modifications as individual events in the germline; F2 = second filial generation in which, according to Mendel, about half of the animals carry the genetic modification in the germline during outcrossing of the F1 animals). If a genetically altered line is classified as impaired, then breeding this line constitutes an animal experiment and must therefore be authorised by the

competent authority. If a genetically altered line is not expected to be impaired, subsequent breeding and keeping must comply with the respective authorisation of the animal housing facility according to § 11 TierSchG.

The term “severity” is not used in the TierSchG. Rather, it is a generic term for the terms “pain, suffering or harm” used in the legal text.

In the jurisprudential literature, the terms “pain”, “suffering” and “harm” are defined as follows, and these definitions are recognised in jurisdiction:

- Pain is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or is described in terms of such damage. - *International Association for the Study of Pain (IASP), 1994*
- Suffering includes all impairments not covered by the term pain which exceed simple discomfort and which persist for a considerable period of time. - *Hirt/Maisack/Moritz, Tierschutzgesetz. 2007, Vahlens Kommentare, p. 81*
- Harm is a condition of the animal which deviates from the normal towards the worse and does not pass quickly. - *Lorz/Metzger, Tierschutzgesetz. 2008, Verlag C.H. Beck, p. 85*

The severity assessment of genetically altered fish currently focuses on the observation of visible alterations in the fish. If new scientific insights into the pain perception of fish are available, they will be taken into account in any future severity assessment updates.

For the purpose of the severity assessment no additional animals are to be bred. The assessment is based exclusively on the observation of the animal. The fish shall not be exposed to any interventions or other manipulations that may induce stress.

Due to the type of genetic modification induced, pain, suffering or harm are not expected in the following fish lines: (1) marker (reporter) lines in heterozygotic breeds, (2) dual expression systems as well as (3) inducible systems that do not lead to induction/expression of the relevant gene, under fish husbandry conditions, that are in accordance with the conditions set forth in a permission pursuant to § 11 TierSchG.

1.2 Scope of application of the forms

The forms are used for the severity assessment of genetically altered teleost fish. The fish species of the assessed line must be specified in more detail in the forms.

The final severity assessment (*Form 3*) is undertaken on the basis of two observation points:

1. Larval stage at the point of independent feeding (*Form 1*)
2. Adult, sexually mature animals (*Form 2*)

The time point of larvae feeding independently depends on the fish species (in the case of zebrafish usually 6 days post fertilisation (dpf) and in Medaka 12 dpf) and can differ greatly depending on the breeding conditions. The time point of sexual maturity also depends on the fish species as well as the housing conditions and for zebrafish is stated to be about 12-16 weeks.

If alterations are observed at a later point, Form 2 and, if applicable, the final severity assessment (Form 3) must be completed again.

The occurrence of impaired fish can depend on the specific housing conditions within a given facility. Thus the housing conditions should be taken into account in the assessment.

1.3 Instructions on severity assessment

- The severity assessment is conducted in the F3 generation at the latest.
- The assessment is based on animal observation within their receptacle (e.g. petri dish, aquarium, tank).
- For the assessment of adult, sexually mature adults, it is not necessary to use the same animals as for the assessment at the larval stage, since it is usually not possible to permanently mark larvae.
- The alterations listed on the two assessment forms should only occur occasionally (0.1 – 1 %) or rarely (0.01 – 0.1 %) in wild type fish, if the fish are kept in a well maintained facility in accordance with § 1 TierSchVersV with a permission pursuant to § 11 TierSchG.
- Forms 1 and 2 remain at the institution and can be inspected by the authorities upon request.
- Form 3 also remains at the institution, but is presented to the authorities if approval for an animal experiment is sought in accordance with § 8 TierSchG.

1.4 Instructions for filling out Form 1 – Assessment of teleost fish - larvae at the time of independent feeding (Annex 1)

Assessment of larvae is carried out exclusively by observing the animal, depending on size, either by microscopy, in a petri dish or in a tank. A representative number of larvae shall be used for the severity assessment, e.g. for zebrafish a reference value of 20 larvae out of at least 2 clutches of eggs is recommended.

Name of species	Please tick where appropriate; specify “others“
Assessed line - Internal name	Name, that is used within the housing facility: if applicable specify the strain number
Assessed line - International name	Necessary only after the line is published. The name should be given according to international standards, e.g. for zebrafish according to the Zebrafish Model Organism Database (ZFIN), if possible including the specification of the allele
Genetic breed	Please tick where appropriate; specify „others“, e.g. three times heterozygote
Type of genetic modification	Brief description of the type of the genetic modification, the used technique, the target structure and, if possible, of the genetic background
Responsible person	Responsible according to §§ 11-13 TierSchVersV for breeding and maintenance or according to § 8 TierSchG for the authorized project in case of an impairment
Location of the line	Institute and room number
Peculiarities of the facility	If different from the norm, e.g. construction activities/noise, breeding regimen (“express breeding”), type of housing, water temperature, pH
Date of spawning	Since the time point of independent feeding can vary depending on the species and breeding conditions, the date of spawning and date of assessment (see below) must be indicated
Number of larvae	Representative number (e.g. for zebrafish 20 larvae out of 2 clutches). An estimated value is sufficient
Alterations <i>Morphology</i> <i>Swimming behaviour</i> <i>Activity</i> <i>Others</i>	Please fill in “0”, if no alterations occurred. If alterations are observed, please specify the number (n) of the affected animals and describe the alterations briefly under notes. Alterations can relate to: e.g. size, skin/scales, fins, any form of swelling, abnormal flexion, heart oedema, unopened swim bladder e.g. position in the water e.g. swimming on the ground or surface, segregation from the swarm Space to specify other alterations
Assessor	Person, who has assessed the clutches
Date of assessment	See date of spawning
Summary of the possible impairment	Please tick where appropriate. The classification is carried out by the assessor or the responsible person. The reasons for the classification can be briefly stated under notes.

1.5 Instructions for filling out Form 2 – Assessment of teleost fish – adult, sexually mature animals (Annex 2)

Assessment of the adult, sexually mature fish shall be conducted by observing the animal in the tank. In general, a separate assessment of the two sexes is not considered necessary. If there are any signs, that the observed abnormalities are sex-specific, the severity assessment of adult, sexually mature fishes has to be done separately for male and female animals.

Name of species	Please tick where appropriate; specify “others“
Assessed line - Internal name	Name, that is used within the housing facility: if applicable specify the strain number
Assessed line - International name	Necessary only after the line is published. The name should be given according to international standards, e.g. for zebrafish according to the Zebrafish Model Organism Database (ZFIN), if possible including the specification of the allele
Genetic breed	Please tick where appropriate; specify „others“, e.g. three times heterozygote
Type of genetic modification	Brief description of the type of the genetic modification, the used technique, the target structure, and, if possible, of the genetic background
Responsible person	Responsible according to §§ 11-13 TierSchVersV for breeding and maintenance or according to § 8 TierSchG for the authorized project in case of an impairment
Location of the line	Institute and room number
Peculiarities of the facility	If different from the norm, e.g. construction activities/noise, breeding regimen (“express breeding”), type of housing, water temperature, pH
Number of animals	At least 7 animals shall be used for the assessment. Should sex-specific alterations occur, at least 7 male and 7 female animals shall be assessed
Age of the animal	In days post fertilization (dpf)
Alterations	<p>Please fill in “0”, if no alterations occurred. If alterations are observed, please specify the number (n) of affected animals and describe the alterations briefly under notes (see information for the according footnote).</p> <p>Alterations can relate to:</p> <p><i>Body structure</i> See footnote 1 <i>Fins/scales/skin/gills</i> See footnote 2 <i>Behaviour</i> See footnote 3 <i>Others</i> Space to specify other alterations, e.g. increased mortality, disturbed fertility</p>
Assessor	Person, who has assessed the adult, sexually mature fish

1.6 Instructions for filling out Form 3 – FINAL ASSESSMENT Teleost fish (Annex 3)

The name of the institution, including the full address, where the animals are kept for the period of breeding. The details of the genetically altered fish line shall be provided according to the information given in Forms 1 and 2 (**Part A**).

The declaration, why it can be ruled out in advance that the animals will suffer any pain, suffering or harm due to their genetic modification, is optional (**Part B**). This serves only as documentation for the responsible person and can be submitted to the competent authority upon request.

The final assessment of genetically altered teleost fish is based on the observations stated in forms 1 and 2, namely at the two developmental stages “larvae at the time of independent feeding” and “adult, sexually mature animals” (**Part C**). If at a later stage alterations are observed which can be related to the genetic modification, form 2 and form 3 (final assessment) have to be filled out again.

In **Part D** it has to be finally judged, if the animal line is expected to suffer from pain, suffering or harm due to its genetic modification. Please tick “no” for impairment, if no alterations were observed (if necessary, it has to be justified why registered alterations are not classified as harmful). In case of impairment, the grade of severity according to Article 15 Para. 1 of the Directive 2010/63/EU (mild, moderate, severe) has to be indicated and justified by naming the most striking alterations. In addition, measures for refinement can be recommended here.

The final assessment shall be carried out, dated and signed by the responsible person for breeding and maintenance of the animals (according to § 11 TierSchG) or for the authorized project (according to § 8 TierSchG). The competent animal welfare officer confirms with his/her signature and date that he/she has taken note of the final assessment (**Part E**). The competent animal welfare body can be contacted for advice in case of contentious issues.

Name of species Zebrafish Medaka other:

Assessed line – Internal name

Assessed line - International name
 (Necessary only after the line is published)

Genetic breed het x het het x wt hom x hom other:

Type of genetic modification

Responsible person

Location of the line (institute and room)

Peculiarities of the facility

Assessment	Clutch 1	Clutch 2	Clutch 3	
Date of spawning	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	
Number of larvae (approx.)	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	
Alterations (number of affected animals)				Notes
Morphology	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>
Swimming behaviour	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>
Activity	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>
Other	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>
Assessor	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	
Date of assessment	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	<input style="width: 80%; height: 20px;" type="text"/>	

Summary of the possible severity Yes No

Notes

Date Name Signature
 (Assessor or responsible person) (Assessor or responsible person)

A	Institution (Full address) <input style="width: 100%; height: 30px;" type="text"/>	Name of species <input type="checkbox"/> Zebrafish <input type="checkbox"/> Medaka <input type="checkbox"/> other: <input style="width: 100%; height: 20px;" type="text"/>
	Assessed line - Internal name <input style="width: 100%; height: 20px;" type="text"/>	Assessed line – International name <input style="width: 100%; height: 20px;" type="text"/>
	Genetic breed <input type="checkbox"/> het x het <input type="checkbox"/> het x wt <input type="checkbox"/> hom x hom <input type="checkbox"/> other: <input style="width: 100%; height: 20px;" type="text"/>	(Necessary only after the line is published) Specification of the publication <input style="width: 100%; height: 30px;" type="text"/>
	Type of genetic modification <input style="width: 100%; height: 30px;" type="text"/>	
B	<u>Reason, why no impairment is expected due to the genetic modification</u> <input style="width: 100%; height: 30px;" type="text"/>	
	This specification is optional. Only needs to be signed by the responsible person.	
C	<u>Summary of the observations stated in forms 1 and 2</u>	
	Form 1: Possible impairment of the larvae: Yes <input type="checkbox"/> No <input type="checkbox"/>	
	Form 2: Number of adult, sexually mature animals <input style="width: 50px;" type="text"/> Age of animals <input style="width: 50px;" type="text"/>	
	Alterations	Number of affected animals (n)
	Body structure	<input style="width: 100%; height: 20px;" type="text"/>
	Fins/scales/skin/gills	<input style="width: 100%; height: 20px;" type="text"/>
Behaviour	<input style="width: 100%; height: 20px;" type="text"/>	
Other <input style="width: 100%; height: 20px;" type="text"/>	<input style="width: 100%; height: 20px;" type="text"/>	
D	<u>Final Assessment</u> <i>(if necessary, use extra sheet)</i>	
	Severity is classified as: none <input type="checkbox"/> mild <input type="checkbox"/> moderate <input type="checkbox"/> severe <input type="checkbox"/>	
	Justification (comprehensible description of the characteristic impairment) <input style="width: 100%; height: 50px;" type="text"/>	
	The following refinement is recommended for reducing the grade of severity: <input style="width: 100%; height: 50px;" type="text"/>	
E	Name, surname of the responsible person <input style="width: 100%; height: 20px;" type="text"/>	Name, surname of the animal welfare officer <input style="width: 100%; height: 20px;" type="text"/>
	City <input style="width: 50%; height: 20px;" type="text"/> Date <input style="width: 50%; height: 20px;" type="text"/>	Taken notice of on (date): <input style="width: 100%; height: 20px;" type="text"/>
	_____ Signature of the responsible person	_____ Signature of the animal welfare officer