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Classification of inducible transgenic and knockout systems according to animal welfare law

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1. General assessment of animal welfare law

1.1. Assessment of animal welfare law in Germany

Animal experiments are procedures or treatments performed in animals for scientific purposes that may be associated with pain, suffering, or harm to the animals[1]. But “procedures” involving the genotype of animals are also described as animal experiments if they might be associated with pain, suffering, or harm to the genetically modified animals[2]. Likewise included under the heading of animal experiments are procedures and treatments that do not directly serve a scientific purpose but are carried out amongst other things for the propagation of organisms[3]. The mating of animals must also be considered an “animal experiment” within the meaning of Germany’s Animal Welfare Act (TierSchG) if this is done with the aim of breeding offspring (“born or hatched”[4]) that carry genetic modifications which could cause pain, suffering or harm. In such cases, this breeding is also to be regarded as an animal experiment within the meaning of § 7(2) TierSchG. Such an “animal experiment” through purely breeding genetically modified animals is considered to be completed when no further observations are to be conducted in the genetically modified offspring and the offspring are also not expected to feel any pain or suffering as a result of these genetic modifications or suffer any lasting damage[5]. The outcome of an experiment in the generation or breeding of a new, genetically modified animal line cannot be reliably predicted even in cases where the influence of the genetic modification can be estimated with a certain degree of probability with regard to the expected phenotype. So, there is a “scientific purpose” here within the meaning of § 7 (2) TierSchG, first sentence, but not within the meaning of the second sentence. Therefore, the generation of a new genetically modified animal line must always be treated as an animal experiment with a scientific purpose in accordance with §8 TierSchG.

Established mutants with impaired phenotype in legislation

With established mutants, the outcome of genetic modification with regard to the phenotype is known. It can be estimated whether such mutants will have to endure genetically induced pain, suffering, or damage as a result. Also (environmental) conditions are known under which such suffering may occur. However, if it has emerged from careful monitoring with the aid of suitable assessment forms[6] and has been documented in a final assessment that pain, suffering, or damage can be ruled out in animals with genetic modifications, no approval or notification is required for further breeding.

Classification of mutants with impaired phenotype

By contrast, if a genetically induced constraint (pain, suffering or damage) cannot be ruled out, can be expected or has been observed in offspring, then any further breeding requires approval. Only in a case as defined under §8a Para. 1 TierSchG, such as tests required by law, or if there is no scientific purpose as referring to the meaning of §7 Para. 2 Sentence 2

[1] § 7 (2) Sentence 1 No. 1 TierSchG
[2] § 7 Para. 2 Sentence 1, No. 3 TierSchG
[3] § 7 Para. 2, Sentence 2, No. 1 TierSchG
[4] § 7 Para. 2, Sentence 1, No. 2 TierSchG
[5] § 7a Para. 5 No. 2 Animal Welfare Act
[6] Annexes 1, 2, 3, 4 to “Dokumentation und Veröffentlichung der Belastungseinstufung für genetisch veränderte Versuchstiere” in June 2013 of the German Federal Institute for Risk Assessment (BfR)

TierSchG the authorities must be solely notified. In cases of offspring with severe existing or anticipated constraints, however, an application must be submitted for approval[7].

1.2. Assessment of animal welfare law in Switzerland

In Switzerland, genetically modified animals can be created within existing registered husbandries by use of recognized methods such as breeding, pronuclear and blastocyst injection. It requires a general approval for the respective procedure (application form G)[8]. These recognized methods must be carried out using a standardized procedure that is as animal friendly as possible[9]. This includes a documentation of the results[8]. New methods may possibly be recognized[10]. Recording the impaired phenotypes of newly created transgenic animals is regulated in Switzerland's Animal Welfare Act (TSchG), in the Animal Welfare Ordinance (TSchV), and in the ordinance of the Federal Food Safety and Veterinary Office (FSVO) on the housing of laboratory animals, the creation of genetically modified animals, and on procedures in animal experiments (Animal Experiments Ordinance [TVV]). It is unequivocally stipulated that, in all newly created strains and lines not sufficiently characterized, regardless of expectations concerning phenotype, a record of impaired phenotypes or absence thereof must be acquired over at least three generations and 100 animals[11] before a particular new strain can be considered to be without constraints. Monitoring covers nest inspections and inspections during weaning[12]. Between these inspections the animals must be observed once a week, which can be combined with the visual inspection of all animals[13] that is conducted three times a week. The first inspection of new-borns must be carried out within five days of birth. The parameters of these checks on phenotypes are precisely defined[14]. As part of the procedure to record phenotypes, data on reproductive success and mortality must be recorded and compared with the data from non-transgenic animals of the same background strain. If a similar constraint is observed in several animals of one line, this must be reported to the competent authority (cantonal veterinary office) within two weeks, using application form M on a provisional basis, as stipulated in Art. 14 TVV[15] and the authority notified of planned measures to mitigate the impairments. A definitive report is submitted either when the impairment is proven or at the latest after 100 animals and three generations[16]. The non-confirmation of a provisionally reported phenotype must also be reported. The breeding of lines with constraint is only possible on the scale required for the planned experiments. If the degree of impairment is substantial, it is necessary to weigh the benefits against the potential harm and undertake measures to mitigate the impairment[17]. The decision on whether to approve further breeding and supporting measures is taken by the cantonal authorities[18].

[7] TierSchG §8a Para. 2 No. 2
[8] TSchG Art. 11, TVV Art. 142, TVV Annex 1
[9] TVV Art. 9 Paragraph 3 and 4
[10] TVV Art. 9 Paragraph 2
[11] TVV Art. 124, TVV Art. 14 Paragraphs 3 and 4
[12] TVV Art. 14 Para. 2 and 15 Para. 2, Art. 14 Para. 1 and 15 Para. 2
[13] TVV Art. 2 Para. 3
[14] TVV Annex 4
[15] TVV, Art. 126 and 145 Para. 1 a TSchV
[16] Art. 126 and 145 Para. 1 a TSchV, TVV Art. 18
[17] TSchV Art.125, TVV Art. 18d and e
[18] TSchV Art. 127

1.3. Assessment of the legal protection of laboratory animals in Austria

According to § 2 Subpara. 1 c) of the Austrian Animal Welfare Act (Bundesgesetz über Versuche an lebenden Tieren, Tierversuchsgesetz, TVG 2012) any use of animals for scientific purposes which is intended to or may result in a genetically modified animal line being created and maintained in a condition that could cause pain, suffering, anxiety or lasting damage is an animal experiment. Therefore, the production of genetically modified animals, regardless of the method or strategy used, constitutes an animal experiment and requires approval by the competent authority. The same applies to the breeding and housing of genetically modified animal lines: since the impact of a mutation on phenotype, regardless of whether this mutation is constitutive or conditional, cannot be reliably predicted and possible constraints of the animal cannot be ruled out, the breeding and housing of any new line requires approval. Projects in the framework of which the line is used must include a phenotype assessment of the line with regard to severity. The requirement to obtain approval no longer applies as soon as the line is established, and the presence of a largely unimpaired phenotype is proven through relevant documentation. As regards the criteria for the phenotype assessment, reference is made to the annex of the "Working document on genetically altered animals"[19]. It must be borne in mind that in all other respects (housing conditions, etc.) the breeding and housing of unimpaired, genetically modified animals remain subject to the legal provisions on the protection of laboratory animals (TVG 2012, Ordinance on Animal Experiments, TVV 2012).

For new lines, including also multiple mutants created through breeding, a detailed assessment of the expected phenotype must be enclosed with the application, on the basis of which the severity is estimated. For lines already established, results of phenotype characterization are recognized from scientific publications. Insufficiently characterized lines are treated similar to newly created lines.

For the approval of animal experiments in Austria and hence also for the production of genetically modified animals and the breeding and housing of impaired lines, a project assessment must be carried out according to § 29 Para. 2 TVG 2012, including a harm-benefit analysis.

2. Inducible transgene expression

Transgenic laboratory rodents play a prominent role in biomedical research. Syngeneic or xenogeneic transgenes are expressed in these animals. Genetically modified animal models are used for specific research in which influence may be exerted experimentally on the timing and the tissue for transgene expression or for the activity of the gene product. In principle, a distinction is drawn between three strategies for regulating transgene expression: (i) the transactivation of transcription via controllable transcription factors, (ii) sequence-specific DNA recombination, which involves genomic modifications and (iii) post-translational control of the function of a gene product. Some frequently used methods are briefly presented below.

Expression is directly controlled either by the activation of promotors with the aid of endogenous transcription factors or by using an expression system that can be artificially activated, for which transcription factors are likewise used in transgene expression. Examples

[19] http://ec.europa.eu/environment/chemicals/lab_animals/pdf/corrigendum.pdf

of promotors that can be regulated by endogenous transcription factors by means of a signal are Mx1 promoter by type 1 interferon (Kühn et al. 1995) or poly(I)/poly(C), *phosphoenol-pyruvate carboxykinase (Pck1)* gene promoter (McGrane et al. 1988) by the protein and carbohydrate content of food and *Hspa1b (Hsb70b)* promoter by heat (Smith et al. 2002). Other systems are based on the transgene expression of controllable transcription factors. Possibly the most frequently used system is the Tet-Off or Tet-On system. In this system, the controllability of the target transgene is achieved by transgene expression of the tetracycline-controlled transactivator tTA (Tet-Off system) (Gossen and Bujard 1992) or of the reverse tetracycline-controlled transactivator rtTA (Tet-On system) (Gossen et al. 1995) (Fig. 1A and B). These transactivators can be placed under control of a tissue or cell-specific promoter. In the Tet-Off system the target transgene, which carries the tetracycline response element *(TRE)* promoter, is switched on by removing the tetracycline, usually doxycycline. In the case of the Tet-On system, this happens by adding doxycycline. Another system is the LightOn model (Wang et al. 2012) in which gene expression is induced by exposure to blue light (Fig. 1C). For this the transcription factor GVAP is expressed, which is dimerized under light and binds to the artificial promoter (Wang et al. 2012).

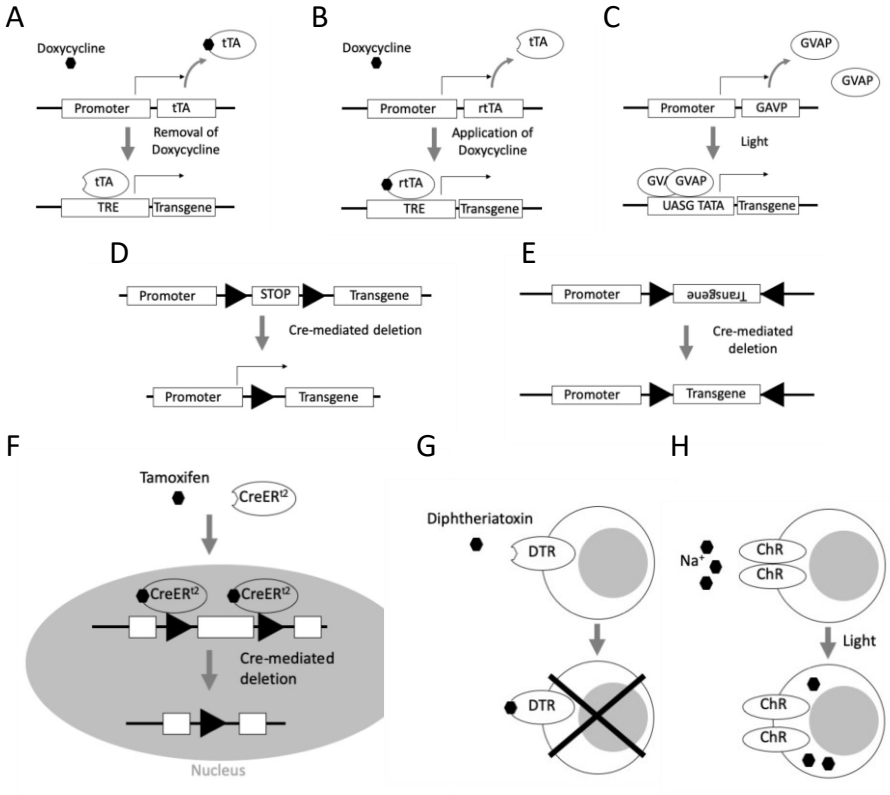

Figure 1: In the Tet-Off system (A) the TRE (tetracycline response element) promoter of the transgene to be regulated is activated by the removal of doxycycline. In the Tet-On system (B) the TRE promoter is switched on by the addition of doxycycline. (C) Dimerization of GVAP by irradiation leads to activation of the UASG promoter element. (D) Cre-mediated deletion of a stop cassette can be used for the controlled activation of a gene. (E) Cre-mediated inversion can switch transgenes on and off, as indicated by the orientation of the word “transgene”. (F) CreER^{T2} can enter the cell nucleus and trigger recombination there only after the binding of tamoxifen. (G) Transgenic expression of a diphtheria toxin receptor (DTR) allows cells to be ablated by the addition of diphtheria toxin. (H) Channel rhodopsin (ChR) is a light-controlled cation channel.

However, gene expression can also be regulated by a recombinase, such as Cre, Dre or FLP recombinase, and their target sequences *loxP*, *rox*, and *frt* (Golic and Lindquist 1989; Sauer and Henderson 1989; Anastassiadis et al. 2009). A stop cassette consisting of transcriptional and translational stop mechanisms is flanked with *loxP* sites and located between promoter and open reading frame. Recombination deletes the cassette, and expression can take place (Fig. 1D). Another possibility for transgene activation consists in bringing a DNA segment flanked with reverse *loxP* sites into transcriptional orientation through recombination mediated by Cre recombinase (Fig. 1E).

3. Inducible conditional mutation of endogenous loci

Conditional knockout systems have found widespread use in the last few years, because they permit a target gene to be inactivated in a particular cell type, tissue or organ of the body or at a set time. It is possible to determine the function of a gene more accurately with these systems than with constitutive knockouts. What conditional knockout have in common is that sequence-specific recombinase systems are used to inactivate the target gene. The most frequently used is Cre recombinase, followed by Dre recombinase and FLP recombinase, with the corresponding target sequences *loxP*, *rox* and *FRT*. The target gene or essential parts of it are flanked by gene targeting, e.g. by *loxP* sites ("floxed"). The transgenic expression of Cre recombinase acts as the second part of the system. When crossed this leads to the floxed target region being cut out (Fig. 1D) (when the orientation of the *loxP* sites is identical). The other recombination systems are used in an analogous way. To inactivate a gene at a given time, the fusion protein of Cre is usually used today as a fusion with a mutated ligand-binding domain of the human oestrogen receptor ($CreER^{T2}$) (Feil et al. 1996; Indra et al. 1999). $CreER^{T2}$ is usually kept in the cytoplasm, bound to heat shock protein (HSP), and, after tamoxifen-induced dislocation of HSP, translocates to the nucleus. Here the recombination of the target gene takes place (Fig. 1F). A further variant of inducible Cre activity can be achieved through the Tet-On system by inducing the expression of Cre recombinase under control of the *TRE* element (Saam and Gordon 1999).

A combination of the systems induces the expression of mutated exons. Here an exon or a gene region is flanked by *loxP* sites and the same region, albeit with e.g., point mutations, introduced behind the *loxP*-flanked region. Now, through Cre activity, it is possible to switch from expression of the wild-type protein to the mutated protein in the endogenous locus (Kraus et al. 2001).

4. Viral and further gene expression systems

The introduction of DNA or RNA into somatic cells by viruses, chemical or physical methods also represents a form of induced transgenic gene expression in the widest sense of the term. Retroviral and lentiviral expression is often used to modify cells of the haematopoietic system and is stable as a result of integration into the genome of the target cells. In this way the entire immune system of modified cells can be built up through the transduction of haematopoietic stem cells following the generation of bone marrow chimeras (Szymczak et al. 2004). Adenovirus can be used for the liver, albeit with expression that is only transient as a rule. In the neurosciences, adeno-associated virus (AAV) (Smith et al. 1995) and rhabdovirus (Wickersham et al. 2007) are often used to introduce transgenes into cells of certain regions

of the nervous system. A physical method of introducing a transgene is the use of hydrodynamic transfection of hepatic cells with a plasmid (Liu et al. 1999). Also included in these systems is DNA vaccination by intramuscular injection. As a rule, both lead to transient expression. Lipofection is a form of chemical introduction of transgenes into cells and can be successfully used in vivo in combination with cavitation (formation and collapse of bubbles in liquids).

5. Inducible activity of transgene products

In systems of this type, the effect of the transgene is obtained not by controlling expression but by chemical or physical inducing factors. Since some of these systems can also be used for transcription control, there is a certain overlapping with the systems already described. The list of such models is long and highly specific for pertinent fields of research.

Cell ablation systems allow certain cells to be killed at a defined point in time. These include the Herpes simplex thymidine kinase system, which leads to the death of dividing cells following the administration of ganciclovir (Bush et al. 1998), and also the diphtheria toxin receptor system (Fig. 1G), in which cells transgenically labelled with the receptor are killed by injection of the Diphtheria toxin (Saito et al. 2001). Using recombination in combination with cell ablation is effective for transcriptional activation of Diphtheria toxin A expression (Ivanova et al. 2005; Brockschnieder et al. 2006). Here, a stop cassette is often deleted by Cre-mediated recombination following the administration of tamoxifen. In optogenetic systems, the activity of nerve cells is regulated by light exposure. The proteins to be mentioned here are Channel rhodopsin (Boyden et al. 2005) and Halorhodopsin (Han and Boyden 2007), which act as cation and chloride channels respectively.

6. Classification of inducible transgenic and knockout systems, as well as other recombination systems in animal welfare law

6.1. Inducible transgenes and German animal welfare regulations

Back in 1996, the German Federal Ministry of Research at the time drew up an information paper on the classifying the production of genetically modified animals under animal welfare legislation. The core statements of this paper (Cramer et al. 1996), which broadly still apply today, are:

a) According to the German Animal Welfare Act (TierSchG), the creation of transgenic or knockout animal strains constitutes an animal experiment and, if the animals in question are vertebrates or (since 2013) cephalopods, is subject to approval by the authorities.

b) The further breeding of such genetically modified animals beyond the second generation did not have to be either reported to or approved by the authorities until the amendment of TierSchG in 2013 but was considered simply a breeding activity as defined in §11 TierSchG. Also, the further breeding of animals that are carriers of genetic modifications with harmful effects on health was and still is permitted, provided this serves a scientific purpose[20]. Animal breeders are still required to take every action to ensure the wellbeing of the animals under

[20] § 11b Para.3 TierSchG

the housing conditions necessary for them. A prerequisite for exercising this duty of care is that all the personnel involved in the care of the animal stock possess adequate expertise[21], especially with regard to the nature and function of specific mutations of the animals. The duty of care also implies adequate veterinary monitoring of the animal stock and, where applicable, any treatments required together with adequate documentation of the measures carried out. As a result of the change in animal welfare law demanded by the European Union in 2010[22] it is necessary also to notify the competent authorities or request approval for the further breeding of genetically modified animals if observations / investigations on their characterization are conducted in the offspring or pain, suffering or damage is expected to be found as a result of the genetic modification.

Aside from these considerations, of course, any experiments in genetically modified animals that could lead to pain, suffering or damage must still be notified to the authorities or approval obtained from the authorities depending on the purpose of the research and the stress on the animals.

The listed key points of the information paper produced by the former German federal ministry of food, agriculture and consumer protection also apply of course to inducible transgenic or knockout animals. The induction measures are assessed under animal welfare law on the basis of the following factors:

A) Purpose of induction: Induction may be performed for a scientific purpose. Induction may, however, also constitute a veterinary treatment of breeders of particular strains or may serve the purpose of preserving a strain of animals. It can thus be considered part of the responsible person's duty of care according to § 11 TierSchG. For example, the Tet-Off system is active without any intervention, but it can be prevented from activation or can be switched off by adding doxycycline as an inductor (see Fig.1). In breeders and in stock animals containing the Tet-Off system, administration of doxycycline can thus be used to prevent the expression of a gene product that is harmful to their health. In this case, the administration of doxycycline is compulsory, and approval or notification is only required if the harmful effect of the gene product cannot be completely prevented by this means or if pain, damage or suffering is triggered in the animals by the doxycycline or the administration procedure itself (injections or the like).

However, there are interpretative approaches that regard such measures as a "refinement", because while they minimize the risk of stress, they do not completely eliminate it. Therefore, they are seen as a reason always to classify any such procedure, e.g. administration of doxycycline in the Tet-Off system, as an animal experiment according to the EU Directive[23]. But in concrete terms, the Directive[23] states that the "elimination of pain, suffering, distress or lasting harm by the successful use of anaesthesia, analgesia or other methods" is no reason for not including the animals concerned within the scope of the Directive, in other words for not regarding them as animals in an animal experiment. However, it is only possible to eliminate or switch off something that exists or is switched on. So, what is meant is a situation in which a noxious agent already exists as a potential cause of pain, fear, suffering or lasting damage

[21] § 3, Para.1, No. 1 and §11 ara.1, No.1 and 2 TierSchVersV, Wilson et al., 1995; Nevalainen et al., 2000; Nevalainen et al., 1999
[22] EU Directive 2010/63EU, amendment of TierSchG and TierSchVersV
[23] EU Directive 2010/63/EU Art. 1 (2) 3rd sentence

and the stress resulting from this is eliminated using appropriate measures. In the case of appropriate doxycycline administration (induction) in mutants using the Tet-Off system, the expression of a harmful gene product is almost certainly prevented until the treatment is discontinued. Consideration of a possible “residual risk” that an impairment may occur is not addressed in the EU Directive, so it has to be assumed that this norm only refers to specific noxious agents already active and actually present in the animal, but not to the hypothetical possibility that a constraint could occur as a result of special circumstances.

B) Impaired wellbeing of animals as a result of the induction per se: It must be established whether the induction, i.e. the administration of e.g. doxycycline (Tet-On) or tamoxifen (CreER^{T2}) per se causes pain, suffering or damage. If the inductors are administered by the parenteral route, it must generally be assumed that an impairment is caused by the induction itself (injection pain). But the administration of inductors via the food or the drinking water may also be classified as stressful if it leads to the food intake being temporarily or permanently impaired.

C) Impaired animal wellbeing as consequences of induction: it must be established whether the induction of a transgenic or knockout system can lead to pain, suffering or damage in the animals concerned. When it comes to inducible transgenic systems, it can be assumed this is so in cases where the target gene product has pathogenic properties. But it can also be caused by harmful effects resulting from the overexpression of a physiological gene product. For example, the liver-specific overexpression of the pro-inflammatory cytokine IFN-γ leads to hepatitis (Toyonaga et al., 1994). On the other hand, the induction of a tissue-specific knockout defect does not necessarily bring with it a constraint for the carrier animals.

D) Performance of other procedures: as summarized in Table 1, the induction of a transgenic or knockout system for scientific purposes through the administration of an inductor must always be reported to or approved by the authority if the induction itself or the consequences thereof may result in stress for the animals (pain, suffering or damage) or if further stressful procedures are carried out. A requirement to report or obtain approval for the induction is only inapplicable in cases where stresses are caused neither by the induction itself nor by its consequences and where no further procedures are carried out.

The holders of animal housing permits according to §11 TierSchG are required to minimize the harmful impacts of mutations in breed animals and stock animals. This duty of care also extends to inducible transgenic and knockout systems. For example, crossings for combining the required transgenes and alleles of an inducible system are to be kept to the absolute minimum necessary if there is any fear of health impairments in the offspring. If there is a possibility of system-specific constraints occurring in breed and stock animals of inducible transgenic and knockout systems, an adequate health monitoring system must be installed and clear criteria defined for the humane killing of impaired animals (criteria for discontinuation). If in the case of inducible transgenic and knockout systems the inductor (e.g. doxycycline with the Tet-Off system for shutting down gene expression) is administered as part of the duty of care (on the part of the housing permit holder as defined in §11 TierSchG), this is not subject to any requirement to report or obtain approval for the procedure if this (veterinary) treatment itself does not cause pain that is more severe than or as severe as the pain of an injection using a cannula. Such a minimal level of stress can be tolerated for administering with the drinking water or food if this is well accepted and ingested voluntarily.

These (veterinary) measures will predominantly affect the Tet-Off system, in which the transgenic system remains in a state of shut-down as a result of doxycycline administration. In this way, the expression of harmful transgenic products, such as those of oncogenes, can be inhibited in breed or stock animals. Cases are also possible, however, in which an improvement can be achieved in the health of carrier animals by activating a Tet-On system. Such a case occurs, for example, in defect mutants when the specific defect can be compensated by the activation of a Tet-On system. The keepers of such genetically modified animals, which can only live free of pain or suffering with appropriate medication, are required to guarantee the animals receive the necessary treatment as demanded. If they want to omit treatment for scientific reasons, they must always obtain approval for the animal experiment respectively notify the competent authority.

According to the new animal welfare legislation, however, the breeding itself is also deemed to be an animal experiment which must be reported or for which approval must be obtained if the genetically modified animals are impaired in their wellbeing despite therapeutic or palliative measures or because there is no knowledge of such measures being administered.

As with all animal experiments, so too with breeding (targeted reproduction of organisms), the purpose and other conditions are pivotal in deciding whether this animal experiment requires approval by or notification of the authority. Animal experiments that require approval are those which serve a scientific purpose and whose result is not sufficiently known, even if the objective is to conduct a duplicate or repeat experiment for a mandatory review of an experimental result that is sufficiently known[24]. Instead of the requirement for approval, however, there is under certain circumstances a duty to notify the authority, e.g. when such experiments are required by law[25]. Notification (20 working days before the start of the experiment; for start of the experiment see Confirmation of receipt[26]) is sufficient also, for example, if the animal experiment is conducted according to proven methods and e.g. for the reproduction of organisms[27].

However, if phenotypes resulting from genetic modification or from a necessary treatment method are to be classified as “severe”[28], an application for the approval of any such breeding must always be obtained before the start of mating. Mating may only be started after approval has been obtained. In the event of considerable pain or suffering that is prolonged and cannot be alleviated, however, the approval may be revoked by the EU Commission[29].

6.2. Inducible transgenes and Swiss animal welfare regulations

Swiss legislation requires a record of impairments for all newly produced or newly imported genetically modified lines. On the basis of this record, a decision is made as to whether a line is without constraint and can continue to be bred without a special permit or may only be housed after approval by the cantonal authorities (see Section 1b). The production of genetically modified animals is not an animal experiment requiring an individual application for

[24] § 8 Para. 1, No. 1 TierSchG
[25] § 8a Para. 1, No. 1 TierSchG
[26] § 36 TierSchVersV
[27] § 8a Para. 1, No. 3 a TierSchG
[28] § 8a Para. 2, No. 2 TierSchG
[29] § 26 Para. 1 TierSchVersV

approval but may be carried out after a simplified approval procedure (application using Form G). In the case of a newly created line, the initial characterization, i.e. also the killing of animals for the analysis of organs or cells, may proceed without a specific approval for animal experimentation. In the case of established lines, however, the killing of animals for the analysis of organs or cells requires approval. The administration of inductors or repressors of transgene expression, such as with the Tet system, may be considered a constraint-mitigating measure after consultation with the cantonal authority. The authority may, however, also require this line to be reported as impaired using Form M. Then the authority will decide on breeding restrictions. With respect to the application for approval of an animal experiment the suffering as the result of a gene-specific phenotype has to be considered together with that of a planned experiment, both, in the prospective classification of the constraint and when weighing the benefits against the harm.

6.3. Inducible transgenes and Austrian animal welfare regulations

Classification of the severity of the prospective impairment of genetically modified animal lines requires in principle the assessment of each individual case, which must also take into account the different conditional alleles of a gene or the specific activation conditions of an inducible system where applicable. A phenotype assessment is thus also undertaken for lines with conditionally inducible mutations whose genetic modification alone is not expected to influence the phenotype.

If the conditional trait of a transgenic locus rests on the use of recombinases or integrases, this usually requires the breeding of a duplicate mutant, resulting in a new line to be assessed. If the activation of the conditional mutation involves the administration of inductors by means of a method that can cause pain, suffering, fear or lasting damage, approval is generally required for this within the framework of a separate research project. The enteral administration of specific medicines as activator or repressor in the drinking water or the food is not subject to any requirement for approval provided it does not compromise food intake and no side effects are to be expected following the administration of these substances. In all other cases, the effect on the wellbeing of the animals and the potential level of impairment are to be assessed depending on the circumstances of the specific individual case (experimental design). In this way, for example, a decision may be reached to dispense with the evaluation of a new mutant with tissue-specific inactivation if the constitutive knockout of this gene has already undergone a comprehensive phenotype characterization that did not show a legal relevant impairment for the animal.

System	Explanation	Constraint without induction	Requirement for approval or notification without induction	Constraint by the induction itself	Requirement for approval or notification with induction
Tet-Off	Administration of doxycycline shuts down gene expression.	Depending on transgene	Depending on transgene	Drinking water: no in the case of acceptance*	In unimpaired transgenic phenotype under treatment: no* In impaired transgenic phenotype under treatment: yes
Tet-On	Administration of doxycycline activates gene expression.	No	No	Drinking water: no in the case of acceptance	No / in impaired transgenic phenotype: yes
LightOn	Induction of gene expression by blue light.	No	No	Light: no, depending on the study	No / in impaired transgenic phenotype: yes
MX-Cre	Induction of the transcription of Cre-recombinase by type 1 interferon or poly-I/poly-C.	No	No	i.p. injection: yes	Yes
HSP70 Promoter	Induction by heat, e.g. by MRI-focused ultrasound.	No	No	Heat: yes	Yes
CreERT2	Induction of Cre activity by binding of tamoxifen.	No	No	Food: no in the case of acceptance Gavage/injection: yes	Food: no Gavage/injection: yes In impaired transgenic phenotype: yes
floxed STOP cassette	Deletion of a STOP cassette by a recombinase (Cre, Dre etc.).	No	No	-	In impaired transgenic phenotype: yes
Inversion	Inversion of a *loxP*-flanked gene segment.	No	No	-	In impaired transgenic / ablation phenotype: yes
loxP, rox flanking	Removal of a gene segment.	No	No	-	In impaired ablation phenotype: yes
Diphtheria toxin receptor	Cell ablation by injection of diphtheria toxin	No	No	i.p. injection: yes	Yes
Channel rhodopsin	Light-induced cation channel.	No	No	Light alone: no With restraint / anaesthesia: yes With phototoxicity: yes	In impaired induced phenotype: yes
Halorhodopsin	Light-induced chloride channel.	No	No	Light alone: no With restraint / anaesthesia: yes With phototoxicity: yes	In impaired induced phenotype: yes
Thymidine kinase	Cell ablation through administration of ganciclovir.	No, occasionally male sterility.	No	Drinking water: no in the case of acceptance i.p. injection: yes	Drinking water: no in the case of acceptance i.p. Injection: yes In impaired transgenic phenotype: yes

Table 1: Classification of various inducible transgenic systems under animal welfare law, *Doxycycline may possibly be administered as part of the duty of care of the animal keeper as specified in §11 or as a breeding measure (CH).

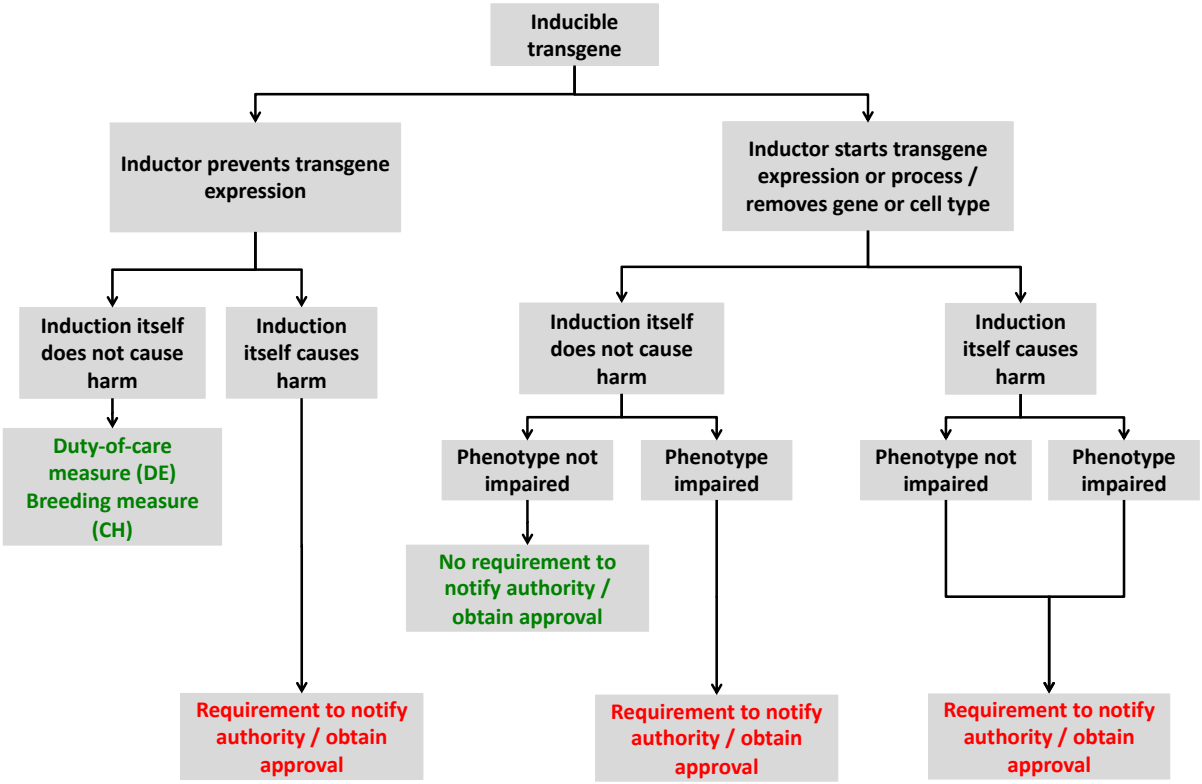

Fig. 2: Flow diagram as decision-making aid to the legal classification of inducible mutants, based on the assumption that a permit has been obtained for the breeding and housing (§11 TierSchG) of (genetically modified) laboratory animals.

7. References

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