Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra

Federal Department of Home Affairs FDHA
Federal Food Safety and Veterinary Office FSVO
Animal Welfare

Technical information Animal experimentation

Severity degrees 1.04

No 1.04_e | 1.9.2018_editorial adjustment of 19.12.2022

514/2014/00547 \ COO.2101.102.5.736231 \ 206.02.02.07

A Introduction

This document provides information for the attention of the cantonal authorities responsible for animal experiments, their advisory committees and all persons concerned with animal experiments.

It is intended to serve as a guideline for careful assessment of the severity of animal experiments and the genetic constraints on the animals, in order to allow correct classification of animal experiments in severity categories. It is also intended to promote efforts to find less constraining animal models and experimental procedures and thus to support the implementation of the 3R principles in the longer term.

1 Legal bases

One of the key statements in Swiss animal welfare legislation is that “no person may improperly subject an animal to pain, suffering, harm or fear, or otherwise violate its dignity” (Art. 4 para. 2 of the Animal Welfare Act AWA, SR 455).

In the field of animal experiments, the Act therefore stipulates that animal experiments entailing constraints on the animal (leading to pain, suffering, harm or fear, significantly impairing the animal’s general condition or otherwise violating its dignity) must be confined to the indispensable need (Art. 17 AWA and Art. 137 AWO). The Animal Welfare Ordinance AWO (SR 455.1) specifies the conditions for approval of animal experiments. An animal experiment entailing constraints can be approved if the experiment does not exceed the indispensable need and if the weighing of constraint against benefits according to Art. 19 para. 4 of the Animal Welfare Act shows that the experiment is permissible (Art. 140 para. 1 AWO).

For each individual animal experiment and for each genetic constraint on the animals, the degree of constraint the experiment imposes on the animals on which it is conducted is assessed qualitatively and, as far as possible, quantitatively, according to standardised criteria. The classification criteria are listed in Section B General description of severity degrees. The legal basis for this is the severity categories in the Animal Experimentation Ordinance, AEO) (Art. 24 and 25, SR 455.163).

2 Objectives and field of application

In the planning and conducting of animal experiments, persons who conduct or cause such experiments to be conducted must be accountable to themselves and to the authorities for the magnitude of pain, harm, fear and suffering inflicted upon the animals. This also applies to any constraints that the animals suffer as a result of genetic modifications. The pain, harm, anxiety and suffering inflicted on the animals should give cause to question and revise new, existing or routinely used animal models or experimental procedures with a view to their refinement (reduction of constraint). In addition, the possibility of whether the experimental question could be answered wholly or partly by means of alternative methods should be re-examined.

Examples are listed in Section C Animal models by specialty areas and severity degrees under the different severity degrees. They give an initial indication of the classification that might be most appropriate for a particular type of procedure. Animal models and experimental procedures not listed in Section C are classified by analogy, based on the expected constraints on the animals. The absence of information on individual specialty areas (---) means that no generally applied animal models or examples of experimental procedures are known of which are classified in these severity degrees.

The severity degree is determined when an application for a licence to conduct animal experiments is submitted, i.e. before the start of the experiment. The application is assigned the maximum severity to which an animal in the planned experiment is expected to be exposed (prospective severity).

After the experiment has been conducted, the severity degrees for individual animals are recorded according to the actual constraints caused, taking into account any unscheduled events, in the interim or final report for animal experiments (Form-C for animal experiments, point 6; explanatory notes regarding Form-C).

Section D Animal models by specific groups of animal species lists the requirements applying to fish used in animal experiments, where a licence is required.

3 Requirements

31 When assigning a severity degree to an animal experiment before it begins, the maximum expected severity is determined by the group undergoing the greatest constraints.

32 The classification of an animal experiment before it begins is made on the assumption that the experiment will be conducted optimally (*lege artis*).

33 Risk factors are to be taken into account in deciding whether to classify experiments in severity degree 0 or 1. Any procedure that is in itself painless, yet difficult to perform flawlessly, is classed as severity degree 1 if this results prospectively in a low degree of constraint.

34 Interventions causing pain must only be conducted with pain relief (Art. 16 AWA). Scientific necessity must be demonstrated in order to use control groups without pain therapy or to withhold pain therapy completely in all animals.
Pain relief (anaesthesia and analgesia) must be administered according to the latest state of the art. The effect of pain treatment must be monitored continuously and improved if necessary. The combination of opioids with anti-inflammatory agents should be considered. Administration of less potent painkillers must be justified.

The partial or complete withholding of pain treatment must result in a higher severity degree.

35 Anxiety and stress caused to the animals must be minimised by means of appropriate measures.

36 Genetic constraints must also be taken into account when determining the severity degree classification and may result in a higher severity degree.

37 The classification criteria listed in Section B General description of severity degrees are used to differentiate between severity categories. The actual severity degree may also be influenced by several other factors, such as:

- the endpoint of the experiment (termination criteria)
- the genetic constraint
- the invasiveness of procedures and interventions
- the quality of pain therapy
- the applied dosages
- the number of times procedures are repeated
- the duration of the experiment

38 If the constraint on the animals is reduced for a model specified in Section C Animal models by specialty areas and severity degree or in Section D Animal models by specific groups of animal species by means of measures during the experiment, the possibility of classifying the experiment in a lower severity category may be considered.

39 If the animal undergoes repeated manipulations during the experiment, the cumulative constraint must be taken into account when assigning the severity degree. The severity degree is not raised if the animal is largely able to recover between repeated manipulations. Repeated blood sampling, as is required in certain models, was taken into consideration in the classification in Section C.

B General description of severity degrees

Constraint caused to animals by interventions or measures in the course of animal experiments (Art. 136 para. 2 AWO, Art. 24 AEO) and caused by genetic modification (Art. 136 para. 2 AWO, Art. 25 AEO) is classed in the following severity categories:

1 Constraint resulting from experimental interventions or measures

Severity degree 0 No constraint: Procedures and actions performed on animals for experimental purposes that do not inflict pain, suffering or harm on the animals, engender fear or impair their general well-being;

Severity degree 1 Mild constraint: Procedures and actions performed on animals for experimental purposes that cause short-term mild pain or harm or a mild impairment of general well-being;

Severity degree 2 Moderate constraint: Procedures and actions performed on animals for experimental purposes that cause short-term moderate or medium to long-term mild pain, suffering or harm, short-term moderate fear or short to medium-term severe impairment of general well-being;

Severity degree 3 Severe constraint: Procedures and actions performed on animals for experimental purposes that cause medium to long-term moderate pain or severe pain, medium to long-term moderate harm or severe harm, long-term severe fear or a severe impairment of general well-being.

2 Constraint due to genetic modifications

Severity degree 0 No constraint: Genetic modifications that do not inflict pain, suffering or harm on the animals, engender fear or impair their general well-being;

Severity degree 1 Mild constraint: Genetic modifications that cause mild pain or harm or a mild impairment of general well-being;

Severity degree 2 Moderate constraint: Genetic modifications that cause moderate pain, suffering or harm, fear or impairment of general well-being;

Severity degree 3 Severe constraint: Genetic modifications that cause severe pain, long-term suffering, severe harm, severe fear or a severe impairment of general well-being.

3 Classification criteria

When classifying a procedure in a severity category, any intervention or manipulation conducted on the animal during the procedure and the genetic constraints on the animal must be taken into account. Each animal experiment must be considered individually.

Factors associated with the procedure include:

- type of manipulation and handling;
- type of pain, suffering, anxiety or (permanent) injury caused by the procedure, its intensity, duration and frequency and the use of multiple techniques;
- cumulative suffering during a procedure;
- restriction of natural behaviour, including restrictions in accommodation, housing and care standards;
- animal species and genotype, genetic constraints;
- degree of development, age and sex of the animal;
- methods to reduce or eliminate pain, suffering and anxiety, including improvements in accommodation, housing, care conditions and habituation;
- if possible, endpoints without pain, suffering or other constraint.

C Animal models by specialty area and severity degree

1 Housing and feeding

1.1 Housing and feeding - Severity degree 0

Housing	Housing meeting the minimum requirements of animal welfare legislation **Examples** Preference studies with various qualities of litter (for improvements in housing conditions) Housing of rats in accordance with animal welfare legislation for ethological observations
Feeding	Feeding with physiological diet without falling short of the minimum requirements of animal welfare legislation or weight loss of up to 5% of initial body weight within 2 weeks in adult animals **Examples** Palatability tests of selected physiological diets or beverages with free access to water Differing feed compositions to test ponderal development in fattening pigs
Deprivation	**Food deprivation**, e.g. overnight, with subsequent compensatory possibilities or euthanasia **Example** Adult mice and rats max. 15 hours

1.2 Housing and feeding - Severity degree 1

Housing	Housing falling slightly short of the minimum requirements of animal welfare legislation, no other deviations from the minimum requirements **Examples** Solitary holding without sensory deprivation max. 7 days Mice and rats in groups in metabolic cages (e.g. grid floor or below the minimum area) with possibility of withdrawal and occupation max. 7 days Dogs in suspension belt for up to 4 hours Holding of dogs in groups without exercise for max. two weeks
Feeding	Unphysiological diet without manifest clinical symptoms or food deprivation for weight loss **Examples** High-fat diet in mice for max. 8 weeks

	Food deprivation in adult animals leading to a weight loss of max. 10% of initial body weight within 2 weeks
Deprivation	**Food deprivation** with subsequent compensatory possibilities or euthanasia **Examples** Adult mice and rats max. 24 hours Adult carnivores max. 24 hours Adult rabbits max. 12 hours Roughage in adult ruminants max. 24 hours **Water deprivation** during dry feeding with subsequent free access to water or possibility of compensation with wet feed in adult animals **Examples** Adult mice and rats max. 15 hours Adult carnivores and farm animals max. 15 hours Adult rabbits max. 6 hours

1.3 Housing and feeding - Severity degree 2

Housing	Housing falling distinctly short of the minimum requirements of animal welfare legislation, or falling slightly short over an extended period **Examples** Isolation housing with sensory deprivation max. 7 days Mice and rats in groups in metabolic cages with possibility of withdrawal and occupation max. 14 days Housing of pigs without occupation max. 2 weeks
Feeding	Unphysiological diet with manifest clinical symptoms or weight loss of max. 15% of initial body weight within 2 weeks in adult animals **Examples** Arteriosclerosis without spontaneous deaths Diabetes and obesity leading to clinically relevant restrictions of organs/organ systems or natural behaviour
Deprivation	Long-term **food deprivation** with subsequent compensatory possibilities or euthanasia **Examples** Adult mice and rats max. 48 hours Adult cats max. 24 hours

	Adult dogs max. 48 hours Adult pigs max. 36 hours Roughage in adult ruminants max. 48 hours Long-term **water deprivation** during dry feeding with subsequent free access to water or possibility of compensation in adult animals **Examples** Adult rats and mice max. 18 hours Adult rats and mice max. 12–23 hours if the period of water deprivation is gradually limited Adult rabbits max. 12 hours Adult carnivores and farm animals max. 18 hours

1.4 Housing and feeding - Severity degree 3

Housing	---
Feeding	Diets leading to a severe clinical picture **Examples** Arteriosclerosis with spontaneous deaths Diabetes and obesity with spontaneous deaths
Deprivation	Long-term **food or water deprivation** **Examples** **Food deprivation** in adult rats >48 hours **Water deprivation** in all adult rats and mice up to 23 hours

2 Experimental reproduction

2.1 Experimental reproduction - Severity degree 0

Breeding and production	Experimental marking and genotyping using non-invasive methods **Examples** Marking with dye Genotyping by hair sample
Germ cells	Collection of germ cells or embryos for experimental purposes from dead parent animals, including females hormonally pretreated to induce superovulation Use of eggs from fish and amphibians if the developing larvae are killed before hatching **Examples** Retrieval of eggs from mice Collection of embryos from rats

2.2 Experimental reproduction - Severity degree 1

Breeding and production	Experimental marking using invasive methods and tissue collection under anaesthesia for the genotyping of adult animals **Examples** Tattooing Ear punching Microchips in rodents and rabbits, experimental tail tip biopsy up to 0.5 cm Toe tip amputation up to the age of 3 weeks
Germ cells	Use of embryos, fetuses or larvae for experimental purposes if they survive the date of birth, hatching or metamorphosis and only mild impairments of the animals are expected

2.3 Experimental reproduction - Severity degree 2

Breeding and production	Experimental interventions to regulate reproduction or for genotyping of adult animals **Examples** Vasectomy Castration

Germ cells	Use of embryos, fetuses or larvae for experimental purposes if they survive the date of birth, hatching or metamorphosis and only moderate impairments of the animals are expected Collection of germ cells or non-viable developmental stages for experimental purposes from living parent animals (all species including Xenopus) **Examples** Mouse strains produced using oncogenes, if the termination criteria are selected accordingly Chemical and radiation-induced mutagenesis for the production of animal lines with defects

2.4 Experimental reproduction - Severity degree 3

Breeding and production	---
Germ cells	---

3 Fetuses and premature animals

3.1 Fetuses and premature animals - Severity degree 0

Sample collection	Euthanasia of fetuses or premature animals in the last third of gestation or development for the collection of body fluids, tissues, organs or body parts by methods in conformity with the Animal Protection Act (including reptiles and birds) Where tissue is collected from fetuses whose mother has been put under deep anaesthesia and is euthanized within 5 to 10 minutes after the onset of anaesthesia, this is classed as euthanasia without pretreatment
Surgical interventions	---
Feeding	---
Reproductive toxicology	---

3.2 Fetuses and premature animals - Severity degree 1

Sample collection	Collection of fluids or tissue resulting in mild impairment of the young animals' further development **Examples** Venous blood collection in premature calves
Surgical interventions	---
Feeding	Restriction of food/energy intake to investigate developmental influences on adult diseases with mild impairments of the foetus or premature animal
Reproductive toxicology	---

3.3 Fetuses and premature animals - Severity degree 2

Sample collection	Collection of fluids or tissue resulting in moderate impairment of the young animals' further development **Examples** Collection of blood from premature small laboratory rodents or rabbits resulting in tissue damage and loss Tail tip amputation for blood collection in small laboratory rodents

Surgical interventions	Intrauterine interventions on the foetus **Example** Intrauterine use of instruments (ECG, EEG recordings) in a ruminant foetus *in utero*
Feeding	Restriction of food/energy intake to investigate developmental influences on adult diseases with moderate impairments of the foetus or premature animal
Reproductive toxicology	Testing of substances for teratological effects resulting in moderate consequences **Examples** Substances causing malformations with moderate constraint but not leading to death

3.4 Fetuses and premature animals - Severity degree 3

Sample collection	Collection of fluids or tissue resulting in severe impairment of the animals' further development or death/abortion.
Surgery	---
Feeding	Restriction of food/energy intake to investigate developmental influences on adult diseases with severe impairments of the foetus or premature animal
Reproductive toxicology	Testing of substances for teratological effects resulting in severe consequences **Examples** Abortion Severe malformations with or without fatal consequences

4 Mutants with a significant clinical pathological phenotype

4.1 Mutants with a significant clinical pathological phenotype - Severity degree 0

Breeding and production	Genetically modified line (spontaneous mutation or genetically altered) used for animal experiments without an impaired phenotype and Genetically modified lines whose well-being is likely to be impaired not by the genetic modification but by the animal experiment **Examples** Reporter lines such as EGFP-expressing lines Conditional and non-induced inducible genetically modified lines
Animal model in the experiment	Mutants (spontaneous mutation or genetically altered) without any clinically manifest diseases, disorders or abnormalities.

4.2 Mutants with a significant clinical pathological phenotype - Severity degree 1

Breeding and production	Genetically modified line (spontaneous mutation or genetically altered) with a mildly impaired phenotype **Examples** Deafness Blindness Dental abnormalities not affecting feed intake Isolated gait abnormalities Mild coordination disorders Immunodeficient animals in SPF housing
Animal model in the experiment	Mutants (spontaneous mutation or genetically altered) with mild clinically manifest diseases, disorders or abnormalities **Examples** Immunodeficient mice in SPF housing Obese mouse without diabetes mellitus Rats with hypertension

4.3 Mutants with a significant clinical pathological phenotype - Severity degree 2

Breeding and production	Genetically modified lines (spontaneous mutation or genetically altered) with a moderately impaired phenotype

	Examples Alzheimer’s disease Parkinson’s disease
Animal model in the experiment	Mutants (spontaneous mutation or genetically altered) with clinically manifest diseases and/or disorders which are compensated by means of appropriate therapeutic measures **Examples** Obese mouse with diabetes mellitus Muscular atrophy Spontaneous diabetes mellitus

4.4 Mutants with a significant clinical pathological phenotype - Severity degree 3

Breeding and production	Genetically modified lines (spontaneous mutation or genetically altered) with a severely impaired phenotype **Examples** Mammary carcinoma Colitis Osteoporosis Kidney diseases Atherosclerosis Aging Arthritis Skeletal abnormalities Motor restrictions Acetylcholinesterase Amyotrophic lateral sclerosis Experimental allergic encephalomyelitis Huntington’s chorea
Animal model in the experiment	Mutants (spontaneous mutation or genetically altered) with severe clinically manifest diseases or disorders without therapy to reduce constraint **Examples** Autoimmune arthritis Knockout animals with severe failure symptoms

	Harlequin mouse with cardiovascular disorders
	Amyotrophic lateral sclerosis
	Frontotemporal lobar degeneration

5 Collection of samples and surgical interventions

5.1 Collection of samples and surgical interventions - Severity degree 0

Collection of samples	Sampling of blood, saliva or urine (non-invasive) without sedation, at intervals and frequencies or in volumes imposing no constraint on the animals (no prolonged restraining measures, no other interventions or previous administrations of test substances) Collection of body fluids, tissues, organs, or body parts under deep general anaesthesia directly followed by euthanasia in animals not previously subjected to any intervention **Examples** Collection of blood samples from the ear vein of the rabbit, twice with an interval of 14 days, 3 ml on each occasion Single non-invasive swabs of body cavities
Surgical interventions	Single injection of small volumes s.c. and i.v. (species-specific), including repeated injections at long intervals (at least 24 hours)

5.2 Collection of samples and surgical interventions - Severity degree 1

Sample collection	*Lege artis* collection of blood, tissue, faeces or urine, with or without sedation, at intervals and frequencies imposing mild short-term constraint on the animals with non-toxic doses of test substances, slightly prolonged reduced housing conditions **Examples** Several blood samples from the tail vein, saphenous vein or sublingual vein in the mouse and rat within 24 hours Skin biopsies Perfusion of small rodents under terminal surgical anaesthesia
Surgical interventions	I.v. or i.p. injections in sedated animals by catheter or tube and substances introduced into the body such as enemas Implants and permanent accesses that can be created and used by means of a minimally invasive (superficial) procedure **Examples** Repeated iv or sc injection of small volumes (species-specific) Insertion of cannulae into peripheral blood vessels Subcutaneous injection of tumour tissue Single subcutaneous implantations of osmotic minipumps and transponders Subcutaneously channelled venous catheters

5.3 Collection of samples and surgical interventions - Severity degree 2

Sample collection	Sampling of blood in volumes and at intervals and frequencies causing moderate short-term constraint on the animals; percutaneous urine collection, collection of tissue samples (cells or fluid), peritoneal washing of naïve animals under general anaesthesia. Sampling of body fluids (in relatively large quantities, in relatively large numbers or at relatively short intervals) after administration of pharmacologically active substances (no toxic doses, no other interventions, no prolonged restraining measures) **Examples** Repeated daily collection of blood samples from the tail vein in rats over five days to determine the course of hormone levels
Surgical interventions	Repeated injections at short intervals (several times within 24 hours) Implants and permanent accesses that have to be created by means of a deep surgical procedure or causing mild long-term constraint on an animal **Examples** Chronic iv catheters, duodenal infusion cannula, hepatic portal vein catheter, gastric tube or chronic intragastric infusion cannula Intraperitoneal or intravenous osmotic minipumps Gavage Telemetry transmitters Implanted iv catheters with pumps in a jacket worn by dogs Implantation of indwelling catheters in ventricles of the brain, or of electrodes in the brain, if the animals retain their freedom of movement Attachment of implants on the intact locomotor apparatus which do not cause any restriction of movement

5.4 Collection of samples and surgical interventions - Severity degree 3

Sample collection	---
Surgical interventions	Implants and permanent accesses that have to be created by means of a deep surgical procedure and causing severe long-term strain on an animal **Examples** Attachment of implants on the locomotor apparatus or other large implants that restrict movement (e.g. dorsal skinfold chamber in mice) Implantation of catheters in the abdominal aorta or bile duct Implantation of an arterial blood-pressure catheter in the aortic arch via the left carotid artery or in the abdominal aorta via the femoral artery Implantation of a combination of a venous and arterial catheter

6 Gnotobiology

6.1 Gnotobiology - Severity degree 0

Immunology of commensals	Experimental animals (except guinea pigs) under gnotobiotic conditions after colonisation of germ-free animals by socialisation with pathogen-free animals which are either monocolonised or have a limited, defined (gnotobiotic) microbiota

6.2 Gnotobiology - Severity degree 1

Immunology of commensals	Colonisation of germ-free animals with non-pathogenic bacteria which, however, may cause a mild temporary pathology in germ-free animals **Examples** Caecal enlargement without further pathologies such as raised diaphragm Colonisation by gastric tube

6.3 Gnotobiology - Severity degree 2

Immunology of commensals	Infection/colonisation with pathogenic bacteria, colonisation with non-pathogenic bacteria which may cause a short-term moderate or long-term mild pathology in germ-free animals **Examples** Infection/colonisation with pathogenic or opportunistic pathogenic bacteria Caecal enlargement with induced pathologies such as female sterility in guinea pigs

6.4 Gnotobiology - Severity degree 3

Immunology of commensals	Infection/colonisation with pathogenic bacteria, colonisation with non-pathogenic bacteria which may cause a short-term severe or long-term moderate pathology in germ-free animals

7 Microbiology and parasitology

7.1 Microbiology and parasitology - Severity degree 0

Infections	Asymptomatic infection with opportunistic micro-organisms (non commensal) or parasites **Examples** Pseudomonas
Blood supply for parasites	---

7.2 Microbiology and parasitology - Severity degree 1

Infections	Infections without symptoms or with short-term mild clinical symptoms **Examples: Bacteria** Induction of localised bacterial dermatitides by various organisms **Examples: Viruses** Subclinical forms of Sendai virus infections in the mouse Spumavirus infection or feline immunodeficiency virus infection **Examples: Parasites** Infestation of carnivores with intestinal stages of cestodes Mild infections with causative organisms of intestinal parasitoses (Giardia, Coccidia, Trichostrongylideae, hookworm) Mild infections with causative organisms of parasitoses of the tissue and blood (Fasciola hepatica, Trichinella, Toxoplasma, Neospora, Plasmodium) Mild to moderate infestations with ectoparasites without repetition (incl. ticks, fleas or flies in rabbits or pigeons)
Blood supply for parasites	The host suffers mild, short-term strain due to the parasites. **Examples** Exposure of the anaesthetised host to mosquitoes Rabbit ear with 20 ticks for 2 hours

7.3 Microbiology and parasitology - Severity degree 2

Infections	Infections accompanied by short-term medium-grade (distinct) or chronic low-grade clinical symptoms **Examples: Bacteria**

	Induction of bacterial vaginitis in the mouse or rat Implantation of a tissue chamber which is subsequently completely colonised by bacteria Models with induced endotoxin shock in laboratory rodents under sedation, with euthanasia while still under sedation **Examples: Viruses** Demonstration of delayed hypersensitivity reaction in choriomeningitis virus infection by footpad swelling Production of seed virus for the preparation of tick-borne encephalitis virus antigen **Examples: Parasites** Infections with pathogenic doses of lungworms (e.g. large lungworm, Dictyocaulus viviparous), tissue parasites (e.g. metacestodes of Echinococcus) and blood protozoa (e.g. trypanosomes (including Trypanosoma brucei, Leishmania and Trypanosoma cruzi), Babesia, Plasmodium)
Blood supply for parasites	The host is either repeatedly infested with the same parasite, or is infested once but with several different parasites, leading to moderate strain on the host The animal is held in a contention system, the parasite burden is low **Examples** Repeated infestation of cattle with horn fly (one infestation per week until decrease in efficacy) Production of ticks on cattle Exposure of a non-anaesthetised host for up to one hour with no possibility of avoidance and with a maximum parasite count of 100 female mosquitoes

7.4 Microbiology and parasitology - Severity degree 3

Infections	Infections characterised by progressive or chronic severe clinical symptoms **Examples: Bacteria** Models with infections for screening new antibiotics Models with induced endotoxin shock in conscious animals Efficacy tests of vaccines in accordance with Ph. Eur. (incl. equine glanders, swine erysipelas) Demonstration of toxin in routine diagnostics and inspection of foodstuffs (Clostridia, tetanus, botulism, blackleg, gaseous gangrene) **Examples: Viruses** Intracerebral infection of the mouse with LCM virus (lymphocytic choriomeningitis) Efficacy tests of vaccines (tests of antigenic efficacy) in accordance with Ph. Eur. (incl. rabies, parvovirus, distemper, influenza, foot-and-mouth disease)

	Examples: Parasites Infections with high doses of Fasciola hepatica in sheep, lungworms, tissue parasites (e.g. metacestodes of Echinococcus), blood protozoa (trypanosomatids, Plasmodium, Babesia spp.) or ectoparasites in immunodepressed animals (incl. mange mites)
Blood supply for parasites	Severe infection with possible wounds **Examples** Massive parasite burden Intestinal parasites in sheep

8 Immunology

8.1 Immunology - Severity degree 0

Transplantations	--- For the severity classification of transplantation with regard to the surgical intervention, see also Section 11 Surgery
Cellular reactions	---
Autoimmune reactions	---
Asthma	---
Immunisation	---
Immunity	---
Inflammation	---
Arthritis	---

8.2 Immunology - Severity degree 1

Transplantations	Interventions causing mild short-term pain or injury and low-grade local changes without disturbances of body function or general condition **Examples** Subcutaneous transplantations of organs without physiological function in the recipient animals Transplantation of mouse hearts subcutaneously behind the ear of recipient mice Transfer of immune cells into a recipient animal with or without mild temporary pathology Donor animals euthanized under deep anaesthesia after the surgical intervention
Cellular reactions	Experiments inducing low-grade local tissue reactions without disturbances of body function or general condition **Examples** Local graft-versus-host reaction Delayed type hypersensitivity (DTH, Jones Mote reaction)

Autoimmune reactions	---
Asthma	**Examples** Bronchoscopy, broncho-alveolar lavage or pulmonary function test in the anaesthetised animal Advanced passive cutaneous anaphylaxis Induction of eosinophilia by repeated intraperitoneal administration of polymyxin B
Immunisation	**Examples** Subcutaneous immunisation with adjuvants not inducing granulomas Intradermal immunisation without adjuvant
Immunity	Application of inactivated bacteria, viruses or parasites (or constituents thereof) without subsequent challenge to test the immune response, with exclusively low-grade short-term local inflammatory reaction (no foot injection) **Examples** Application of vaccines (incl. equine influenza, parvoviroses, equine virus abortion) for subsequent testing of immunogenicity Validation of a viral vaccine in field trials
Inflammation	Experiments inducing low-grade local tissue reactions without disturbances of body function or general condition **Examples** Arachidonic acid test on the mouse ear
Arthritis	Collagen-II-induced arthritis or adjuvant arthritis with low-grade local tissue changes without clinical symptoms such as redness or swelling of a toe or foot

8.3 Immunology - Severity degree 2

Transplantations	Transplantations of organs without physiological function in the recipient animals (with the exception of subcutaneous localisation) **Examples** Second heart transplantation into the abdominal cavity Transplantation of islet cells under the kidney capsule Models with skin grafting, without severe restriction of movement

	Transfer of immune cells causing transient clinical disease in the recipient animal
Immunity	Application of inactivated bacteria, viruses or parasites (or constituents thereof) without subsequent challenge to test the immune response, with significant inflammatory reaction
Cellular reactions	Experiments inducing medium-grade local tissue reactions with transient disturbances of body function or general condition
Autoimmune reactions	---
Asthma	**Examples** Models without respiratory distress Accumulation of leukocytes in the lungs after inhalation of allergens and inflammatory mediators in the sensitised animal Accumulation of leukocytes in the peritoneum after intraperitoneal administration of allergens and inflammatory mediators in the sensitised animal Whole-body plethysmography
Immunisation	**Examples** Subcutaneous immunisation of rabbits, mice, rats or guinea pigs using Freund's complete adjuvant (or adjuvant with comparable high mineral oil content), no administration into the foot Intradermal immunisation with or without adjuvant Immunisation with small amounts of antigen, directly under the splenic capsule (operation) or into a lymph node, under general anaesthesia Tuberculin reaction after intracutaneous injection into a foot
Inflammation	Inflammation causing medium-grade local tissue reactions with transient disturbances of body function or general condition **Examples** Induced peritoneal macrophages for longer than 3 days (with administration of painkillers during the experiment) Air pouch model in the rat Screening of anti-inflammatory agents in mouse strains with spontaneously occurring autoimmune disease (MRL lpr/lpr mice) All models with acute paw oedema with the exception of CFA application using paw volume as measurement criterion and with <6 hours test duration Psoriasis model: Oxazolone-induced DTH (delayed type hypersensitivity) in the ears of mice (redness, oedema, inflammation)

Arthritis	Experiments inducing medium-grade local tissue reactions with transient disturbances of body function or general condition **Examples** Randall-Selitto test Adjuvant arthritis with euthanasia of animals if weight-bearing capacity of a leg is restricted Collagen-II-induced arthritis with symptoms in more than one toe on a foot Euthanasia of the animal within 14 days after inducing the arthritis

8.4 Immunology - Severity degree 3

Transplantations	Transplantation of organs with physiological function in the recipient animal, the failure of which leads to severe strain **Examples** Rejection of limb after allotransplantation Kidney transplantation Pancreas transplantation
Cellular reactions	Experiments inducing generalised tissue rejection reactions **Examples** Generalised graft-versus-host reactions
Autoimmune reactions	Experiments inducing generalised inflammatory reactions in the body **Examples** Acute and recurring experimental allergic encephalomyelitis Mercury-induced glomerulonephritis Experimental uveoretinitis Transmitted rheumatoid arthritis
Immunity	Application of inactivated bacteria, viruses or parasites (or constituents thereof) with subsequent challenge (immunogenicity tests) to test the immune response **Examples** Influenza leading to severe clinical symptoms such as a drop in body temperature to 32 degrees or below in mice LCMV i.c. (lymphocytic choriomeningitis virus)
Asthma	**Examples**

	Triggering of anaphylaxis Acute Respiratory Distress Syndrome (triggering of endotoxin shock in the conscious animal)
Immunisation	Any immunisation of animals with autologous tissue that leads to autoimmune disease if the experiment is not terminated prematurely, EAE model with MOG peptide immunisation
Inflammation	Inflammation causing medium to long-term moderate or severe pain and suffering as well as injury **Examples** Pertussis pleuritis in rats and mice Relapsing encephalomyelitis model, without euthanasia of the animals during the first episode Models of acute hind paw oedema including CFA injection <7 days DSS- and TNBS-induced colitis T-cell transfer for colitis induction
Arthritis	Arthritis with severe clinical symptoms and/or long-term constraint after induction of arthritis **Examples** Adjuvant arthritis with inflammation and/or swelling of an entire paw or ankylosis Collagen-II-induced arthritis with clinical symptoms Carragheen arthritis model Arthritis induction in inbred mouse strains with Borrelia spirochetes Autoimmune arthritis Arthritis induced by xenogenic or autologous collagen-II with symptoms such as inflammation and/or swelling of an entire paw

9 Pharmacology and toxicology

9.1 Pharmacology and toxicology - Severity degree 0

Pharmacokinetics	---
Toxicology	---
Batch testing	---

9.2 Pharmacology and toxicology - Severity degree 1

Pharmacokinetics	**Examples** Administration of substances and blood sampling in freely mobile rats and mice at intervals of several minutes to hours by venous catheter, with replacement of the sampled blood volumes by plasma expander or donor blood, with or without a stay in the metabolic cage <7 days Steady state measurement by infusion in freely mobile rats by vascular catheter, without a stay in the metabolic cage Steady state measurement by infusion in the dog, in a suspension belt for <4 hours, with or without a bladder catheter Application of test substance in non-toxic doses followed by euthanasia of the animals (drug-receptor binding *ex vivo*, tissue level by autoradiography)
Toxicology	Tolerance studies which are expected to cause transient mild reactions, local or systemic, and do not cause substantial strain on the animals owing to the mode of administration or sample collection routes **Examples** Genetic toxicity tests
Batch testing	Studies which are expected to cause transient mild reactions, local or systemic, and cause only mild strain on the animals owing to the mode of administration or sample collection routes **Examples** Application of vaccine batches for subsequent testing of immunity in accordance with Ph. Eur.

9.3 Pharmacology and toxicology - Severity degree 2

Pharmacokinetics	Studies which are expected to cause transient moderate or chronic mild reactions, local or systemic, and do not cause severe strain on the animals owing to the mode of administration or sample collection routes

	Examples Administration of test substance and blood sampling in freely mobile rats at intervals of several minutes to hours by venous catheter, without replacement of the sampled blood volumes by plasma expander or donor blood, with or without a stay in the metabolic cage for max. 14 days Measurement of substance concentration in the brain or cisterna magna in the rat, using the microdialysis method or chronically implanted cannula in the cisterna magna Freely mobile rat with bile fistula <4 days Skin resorption in the mini pig (<30 days metabolic cage, indwelling catheter)
Toxicology	Tolerance studies which are expected to cause transient moderate or chronic mild reactions, local or systemic, and do not cause severe strain on the animals owing to the mode of administration or sample collection routes (no lethality expected) **Examples** Acute toxicity tests and acute tolerance tests in dogs Subacute and subchronic toxicity tests Range finding studies with rodents, rabbits and dogs Bioaccumulation tests in fish Chronic toxicity tests/carcinogenicity tests with oral administration of test substances Reproductive toxicology tests Toxicokinetics with oral administration of the test substance and collection of body fluids
Batch testing	Studies which are expected to cause transient moderate or chronic mild reactions, local or systemic, and do not cause severe strain on the animals owing to the mode of administration or sample collection routes

9.4 Pharmacology and toxicology - Severity degree 3

Pharmacokinetics	Studies causing long-term moderate to severe pain and injury **Examples** - Rats with bile fistulas or lymph fistulas with significantly restricted freedom of movement
Toxicology	Tolerance studies that cause long-term moderate to severe pain and injury or are expected to cause death **Examples** - same test models as under SG2 with expected severe effects

Batch testing	Batch testing that causes long-term moderate to severe pain and injury or is expected to cause death **Example** - Abnormal toxicity - Efficacy tests of vaccine batches (tests of antigenic efficacy) - Tests of the biological activity of growth hormone in hypophysectomised rats

10 Pain

10.1 Pain - Severity degree 0

Pain	---

10.2 Pain - Severity degree 1

Pain	Experiments causing mild short-term pain at the pain detection threshold with slight tissue swelling **Examples** Plantar test, Von Frey filament test or Randall-Selitto test without inflammation or neuropathy (Hargreaves method) Hot plate test or cold plate test Tail flick test Tail immersion test

10.3 Pain - Severity degree 2

Pain	Experiments causing short-term moderate pain or medium to long-term mild pain **Examples** Formalin test inducing nocifensive reactions lasting not longer than 60 minutes Local subcutaneous capsaicin injection Paclitaxel and toxin-induced neuropathy Writhing test with < 0.2 ml 2% acetic acid or 0.4 ml 1% aqueous acetic acid All models with acute paw oedema (e.g. subcutaneous zymosan A) with withdrawal as measurement criterion Chemically induced sub-acute pain model Writhing test with 0.25 ml aqueous suspension of phenyl-p-benzoquinone 0.02%

10.4 Pain - Severity degree 3

Pain	Experiments causing severe pain with severe impairment of mobility, with dehydration and weight loss or automutilation and autotomy behaviour **Examples** Bone cancer pain Chronic constriction injury (CCI) of the sciatic nerve, spinal nerve ligation and spared nerve injury (SNI) without autotomy

11 Surgery

11.1 Surgery - Severity degree 0

Surgery	---
	For the severity classification of the immunological aspects of transplantation, see also Section 8 Immunology

11.2 Surgery - Severity degree 1

Surgery	Minor surgical and other interventions (minor tissue trauma) under general or local anaesthesia with minor postoperative pain, suffering and impairment of general condition
	Examples
	Skin biopsies
	Insertion of cannulae into peripheral blood vessels Subcutaneously channelled venous catheters
	Surgery under general anaesthesia without recovery
	Vasectomy in mice and rats

11.3 Surgery - Severity degree 2

Surgery	Surgical and other interventions on animals under general anaesthesia with moderate postoperative pain, suffering or disturbance of general condition
	Examples
	Laparotomy, laparoscopy such as ovariectomy, hysterectomy, unilateral nephrectomy, splenectomy, creation of gastric fistula in rat and dog
	Xenopus oocyte recovery max. 2x, 2nd collection terminal
	Craniotomy
	Orchidectomy and neutering in the female animal
	Lymphadenectomy
	Thyroidectomy, hypophysectomy with hormonal substitution
	Attachment of implants on the intact locomotor apparatus
	Orthopaedic surgery with effective stabilisation and wound care
	Plastering of limbs for the study of muscular atrophy
	Organ transplantation with effective treatment of rejection

11.4 Surgery - Severity degree 3

Surgery	Surgical and other interventions under general anaesthesia with severe or chronic postoperative pain, suffering or disturbance of general condition **Examples** Creation of unstable fractures Back, pelvic and intervertebral disc surgery Joint transplantation Induction of infections in bone and joint structures Transplantation of a functional internal organ Intestinal resection Hepatectomy 86% One kidney, two clips Opening of the chest, thoracotomy, intercostal thorax access, thoracoscopy Laparotomy (in the dam) with *in utero* electroporation in the last third of gestation with subsequent carrying to full term and birth of the litter Attachment of implants on the locomotor system if this leads to a restriction of movement Restriction of function

12 Heart and circulation

12.1 Heart and circulation - Severity degree 0

Heart	**Examples** Monitoring ECG by non-invasive methods, leading to no or minimal impairment in habituated animals
Circulation	**Examples** Oxygen measurement using pulse oximeter

12.2 Heart and circulation - Severity degree 1

Heart	**Examples** Models with ECG recordings in the conscious dog after administration of test substances in non-toxic doses Preterminal infarction models in the anaesthetised animal and euthanasia while under anaesthesia Reperfusion models in the anaesthetised animal and euthanasia while under anaesthesia
Circulation	**Examples** Models with invasive arterial blood-pressure measurements by means of catheters previously inserted under anaesthesia in practically freely mobile animals, with the exception of abdominal arterial catheters Models with non-invasive blood-pressure measurements in conscious mice, rats or using the cuff method

12.3 Heart and circulation - Severity degree 2

Heart	**Examples** Models with telemetric heart-rate measurements in the conscious animal by means of catheters/transmitters implanted in the abdominal cavity, without clinical insufficiency
Circulation	

12.4 Heart and circulation - Severity degree 3

Heart	Models resulting in clinical insufficiency or interventions that may cause severe pain, extreme anxiety or death of the animal **Examples** Testing of cardiac support devices

	Induction of clinically manifest cardiac insufficiency, myocardial infarction or endocarditis Models with telemetric blood-pressure measurements in the conscious animal by means of catheters/transmitters implanted in the abdominal cavity Models with experimentally induced hypertension in the animal
Circulation	**Examples** Goldblatt rats with organ failure DOCA rats Blood-pressure measurements in pulmonary vessels MCAO (middle cerebral artery occlusion)

13 Endocrinology

13.1 Endocrinology - Severity degree 0

Endocrinology	---
Bone metabolism	**Examples** Administration of vital stains with known innocuous properties in the drinking water or food for the study of dental or osseous development *ex vivo*
Glucose metabolism	Fasting (water *ad libitum*) of adult animals of at least normal weight for max. 3 hours followed by a small blood sample **Examples** Fasting Plasma Glucose (FPG) test
Exercise	Testing the coordination skills of animals **Examples** Grip test Voluntary wheel exercise, e.g. with defined resistance variation and/or variable rod distances with or without positive reinforcement

13.2 Endocrinology - Severity degree 1

Endocrinology	Administration of the test substance with subsequent blood sampling once or at intervals and frequencies imposing only mild short-term constraint, or euthanasia for the determination of hormone concentrations in the blood
Bone metabolism	**Examples** Determination of ossification or bone resorption *ex vivo* in the rat, after repeated administration of the test substance p.o. Determination of ossification in the mouse by marking of matrix synthesis, after repeated substance administration p.o.
Glucose metabolism	Test methods with applications and blood samples at intervals and frequencies imposing mild short-term constraint on the animals, under slightly prolonged reduced housing conditions **Examples** Measurement with injection or gavage of a metabolic substrate or hormone followed by successive blood sampling over a few hours Oral or intraperitoneal glucose tolerance test (OGTT, IGTT) Intraperitoneal insulin sensitivity test (IPIST)

Exercise	Short-term forced exercise causing mild strain, without overexertion and without electrical stimulation **Examples** Treadmill exercise using air-puff Calorimetric wheel, after adequate adaptation to the experimental situation Wheel exercise causing moderate strain, e.g. with defined resistance variation and/or variable rod distances and with negative reinforcement

13.3 Endocrinology - Severity degree 2

Endocrinology	Models giving rise to clinically manifest endocrine disturbances in the animal, with suitable treatment **Examples** Hypophysectomy Adrenalectomy Thyroidectomy Parathyroidectomy Spontaneous diabetes mellitus with clinical findings Obese mouse with diabetes mellitus Lesions of the vagus nerve Streptozocin
Bone metabolism	**Examples** Ovariectomised rats for induction of bone-matrix loss
Glucose metabolism	Test methods with applications and blood samples at intervals and frequencies imposing moderate short-term constraint on the animals, under slightly prolonged reduced housing conditions Examples Euglycemic clamps Hypoglycemic clamps with subsequent euthanasia
Exercise	Forced exercise causing moderate strain without overexertion **Examples** Treadmill exercise with electrical stimulation up to max. 1mA/sec. without exhaustion

13.4 Endocrinology - Severity degree 3

Endocrinology	Models giving rise to severe clinically manifest endocrine disturbances in the animal (decompensation) without suitable treatment (hormone substitution) **Examples** Hypophysectomy Adrenalectomy Thyroidectomy Parathyroidectomy Alloxan diabetes Hyperthyroidism Hypercorticism
Bone metabolism	Long-term models giving rise to severe skeletal deformations or fractures in the animal
Glucose metabolism	---
Exercise	Examples Treadmill exercise to exhaustion by means of electrostimulation VO_2 max or endurance test on a treadmill

14 Tumours

14.1 Tumours - Severity degree 0

Tumours	---

14.2 Tumours - Severity degree 1

Tumours	Models with subcutaneous endogenous tumours if the experiment is terminated before the tumour (owing to its size and location) leads to an impairment of general condition and no cytostatics are administered. **Examples** Passage of primary tumours and tumour cell lines Breeding and maintenance of transgenic or knock-out models of cancer

14.3 Tumours - Severity degree 2

Tumours	Models with induction or transplantation of tumours or with spontaneous tumour development, which are treated with experimental therapies. Experiments are terminated before cancer cachexia or other progressive lethal disease or clinically manifest functional (incl. endocrine) or behavioural disturbances (due to the size, location or other properties of the tumour, or to metastasis) occur in the animal. Weight loss max. 15%. **Examples** Tests of experimental therapies in transplantation models of cancer and in transgenic or knock-out models of cancer

14.4 Tumours - Severity degree 3

Tumours	Models with induction or transplantation of tumours, or with spontaneous tumour development, which cause cancer cachexia or other progressive lethal disease, or which are not discontinued before clinically manifest functional (incl. endocrine) disturbances (due to the size, location or other properties of the tumour, or to metastasis) occur in the animal. Weight loss max. 20%. **Examples** Experiments with dose escalation Tumour therapy models with survival endpoints

15 Neurology and behavioural biology

15.1 Neurology and behavioural biology - Severity degree 0

Behavioural observations	Exclusively observation of the animals or collection of data by other non-invasive methods (no protracted coercive measures, no restricted holding conditions, no interventions or administration of test substances) **Examples** Orientation test in the hamster Optomotor reflex/virtual cliff/visual discrimination tests SHIRPA: Primary screen Dark-light box with unrestricted choice Ultrasound vocalisation recording Digging and burying test
Conditioned avoidance behaviour and conflict tests	---
Social deprivation	---
Exposure to overstimulation	---
Pharmacologically induced behaviour	---
Convulsions	---
CNS lesions	---
Ischaemias	---
Leads	---

15.2 Neurology and behavioural biology - Severity degree 1

Behavioural observations	Observation of the animals or collection of data by other non-invasive methods after administration of pharmacologically active test substances (in non-toxic doses, no other interventions, no protracted coercive measures) **Examples**

	Determination of the effects of substances in the open-field test, labyrinth tests including Morris water maze, or the staircase test
Conditioned avoidance behaviour and conflict tests	Models with stimuli/noxae which are not associated with anxiety and which the animal can successfully avoid **Examples** Passive avoidance test Active avoidance test with stimuli intensity <0.5 mA for 1s Gfeller-Seifert conflict test and Vogel water lick test with stimuli intensity <0.5 mA for 1s Startle response
Social deprivation	Deprivation of social partners (excluding separation of parents and offspring) with olfactory, visual and acoustic contact with conspecifics **Examples** Solitary housing of adult rats or adult female mice for max. 7 days
Exposure to overstimulation	Chronic mild stress **Examples** Models with frequent alteration of environmental conditions in the rat or mouse Repeated holding of laboratory rodents in a small box for one hour Circadian rhythm models
Pharmacologically induced behaviour	---
Convulsions	Convulsion experiments which lead to immediate loss of consciousness (complete convulsion), if the animals do not regain consciousness or are euthanased beforehand **Examples** Maximum electroshock
CNS lesions	Suppression of defined nuclei or pathways under anaesthesia, without modification of the animals' general condition (including feeding/sleeping/social interactions/anxiety) **Examples** Genetically-modified animal models of CNS disorders
Ischaemias	Production of micro-infarcts by established methods, if leading exclusively to short-lasting functional disturbances **Examples** Injection of radiolabelled microspheres (micro-embolism or multi-infarct model)

	Rose Bengal model with activation by irradiation
Implanted probes	Preterminal implantation of probes under anaesthesia
Visual system	**Examples** Electroretinography (ERG) using a circular or filament electrode apposed on the cornea surface of anaesthetised animals Pupillary light reflex measurement in anaesthetised animals Imaging (non-invasive fundus visualisation using a scanning laser ophthalmoscope (SLO), retinal camera or optical coherence tomography (OCT)) in anaesthetised animals Intraocular injection (subretinal or intravitreous) in anaesthetised animals Laser-induced neovascularisation model

15.3 Neurology and behavioural biology - Severity degree 2

Behavioural observations	---
Conditioned avoidance behaviour and conflict tests	Models with stimuli/noxae which are briefly associated with moderate pain, suffering or anxiety and which the animals can successfully avoid, or which are associated with mild pain, suffering or anxiety which the animals cannot avoid **Examples** Passive avoidance test Active avoidance test with stimuli intensity >0.5 mA for 1s Gfeller-Seifert conflict test and Vogel water lick test with stimuli intensity >0.5 mA for 1s Shuttle box Fear conditioning
Social deprivation	**Examples** Contact call in chicks Separation of pair-bonded hamsters
Exposure to overstimulation	---
Pharmacologically induced behaviour	Models with induction of complex modifications in behaviour or physiology **Examples** Induction of a flight reflex

	Oxotremorine test (tremor and salivation) Reserpine catalepsy Cocaine Amphetamine hyperactivity 5-HTP stereotypies Apomorphine climbing Apomorphine hypothermia Reserpine hypothermia
Convulsions	Convulsion experiments which do not lead to immediate loss of consciousness, if the animals do not regain consciousness or are euthanased beforehand **Examples** Petit-mal model
CNS lesions	Suppression of defined nuclei or pathways under anaesthesia, with moderate modification of the animals' general condition (including feeding/sleeping/social interactions/anxiety) **Examples** Unilateral 6-OHDA model of Parkinson's disease (Ungerstedt model) Reversible suppression of brain regions by hypothermia Viral models of genetic disorders Lesions of the corticofrontal lobe Suppression of an efferent motor pathway Viral models of genetic disorders Genetically-modified animal models of CNS disorders Electrically or chemically induced CNS lesions
Ischaemias	Production of ischaemias under anaesthesia by established methods, if the animals show no marked functional disturbances after regaining consciousness **Examples** Levin model in the rat Bilateral carotid ligature in the rat for 30 minutes Pusinelli model for ≤20 minutes Short-term normobaric hypoxia in mice Bilateral carotid ligature in the gerbil for 5 to 30 minutes (depending on the strain)

Implanted probes	Models with chronically implanted catheters/electrodes (including wireless technology) in the skull **Examples** Repeated EEG recordings in conscious rats or mice Cerebroventricular cannulae in the rat for direct, repeated administration of the test substance into the brain Collection of cerebrospinal fluid via cannula in the rat

15.4 Neurology and behavioural biology - Severity degree 3

Behavioural observations	---
Conditioned avoidance behaviour and conflict tests	Behavioural experiments causing severe strain **Examples** Forced swim test (Porsolt test) in small rodents Audiometry in the conscious mouse Chronic social instability Chronic social defeat
Social deprivation	Total, permanent social isolation (incl. olfactory, optical and acoustic) of individuals of gregarious vertebrate species Solitary housing of young animals up to normal weaning age
Exposure to overstimulation	Stress models without habituation (adaptation) of the animal to the stimulus **Examples** Models with frequent changes in the social surroundings in the rat or mouse Experimental models in which laboratory rodents are exposed to noise, foot shocks, cold water and immobilisation over 3 weeks, without any rhythm recognisable to the animal Depression models
Pharmacologically induced behaviour	**Examples** Valproic acid animal model of autism
Convulsions	Convulsion experiments which do not lead to complete loss of consciousness (incomplete convulsions), or if the animals regain consciousness after the cessation of the convulsions **Examples**

	Administration of spasmogenic doses of PTZ, NMDA, picrotoxin, yohimbine, strychnine or kainate
CNS lesions	Suppression of defined nuclei or pathways under anaesthesia with impairment of the animals' general condition (including feeding/sleeping/social interactions/anxiety) **Examples** Models with ablation of relatively large areas of the cerebral cortex
Ischaemias	Production of ischaemias under anaesthesia, if the animals show considerable functional disturbances after regaining consciousness **Examples** Occlusion of the A. cerebri media in the rat and mouse (MCAO) Permanent unilateral carotid ligature in the gerbil All models of cerebral ischaemia with ischaemic episodes lasting >15 minutes (unless specially mentioned beforehand)
Implanted probes	Models with chronically implanted catheters/electrodes (including wireless technology) in the skull, with additional strain **Examples** Head fixation with water deprivation

16 Physical impact

16.1 Physical impact - Severity degree 0

Whole-body irradiation	---
UV irradiation	---
Ultrasound	---
Electro-magnetic radiation	---
Effects of heat	---
Effects of cold	---
Chronic hypoxia	---

16.2 Physical impact - Severity degree 1

Whole-body irradiation	Irradiation or chemotherapy resulting in immune-incompetence of limited duration with subsequent spontaneous reconstitution Irradiation energy 400–450 rad, depending on strain
UV irradiation	UVA and UVB irradiation of mice Irradiation energy: UVA: 50–100 J/cm^2, UVB: 50–500 mJ/cm^2, irradiation time 2–15 minutes
Ultrasound	Measurements in sedated animals with i.v. or i.p. injections of substances **Examples** Testing of contrast agents by means of sample echography
Electromagnetic radiation	NMR (nuclear magnetic resonance) measurements in anaesthetised animals
Effects of heat	---
Effects of cold	---
Chronic hypoxia	---

16.3 Physical impact - Severity degree 2

Whole-body irradiation	Irradiation or chemotherapy with a lethal dose with successful reconstitution of the immune system and total lymphoid destruction **Examples** Partial irradiation with immobilisation of the animal using a fixation device Whole-body irradiation with successful reconstitution of the immune system, if animals are kept in IVC cages with antibiotic cover (irradiation energy 450–900 rad, depending on strain)
UV irradiation	---
Ultrasound	---
Electromagnetic radiation	NMR (nuclear magnetic resonance) measurements under sedation
Effects of heat	**Examples** Induction of small, localised first or second degree burns under anaesthesia with subsequent pain management for topical treatment with test substances, max. 10% body surface area
Effects of cold	Mice and other small rodents at +4°C to reduce body core temperature to 31°C maximum for metabolic studies, for max. 4 hours on one occasion, free access to water. Rewarming on a heating pad.
Chronic hypoxia	**Example** Mice in oxygen tent (100% O_2) with gradual lowering of O_2 concentration within 6 hours to the final O_2 concentration of max. 8%

16.4 Physical impact - Severity degree 3

Whole-body irradiation	Irradiation or chemotherapy with a lethal dose without reconstitution of the immune system
UV irradiation	---
Ultrasound	---
Electromagnetic radiation	NMR (nuclear magnetic resonance) measurements in the conscious, restrained animal

Effects of heat	Induction of small, localised first or second degree burns under anaesthesia with subsequent pain management for topical treatment with test substances, max. 20% body surface area
Effects of cold	Mice and other small rodents at +4°C to reduce body core temperature to 31°C maximum for metabolic studies, for longer than 4 hours on one occasion, free access to water. Rewarming on a heating pad
Chronic hypoxia	Mice in oxygen tent (100% O_2) with gradual lowering of O_2 concentration within 2 hours to the final O_2 concentration of max. 8%

D Animal models by specific groups of animal species

17 Fish in breeding facilities and laboratory animal facilities

17.1 Fish in breeding facilities and laboratory animal facilities - Severity degree 0

Physical influences and husbandry	Manipulation of temperature, photoperiod, water chemistry or stocking density etc., to which the fish can adapt without significant impairment **Examples** Addition of inert markers to water Gradual changes in chemical and physical water parameters
Feeding	Modified diets to which the fish can adapt without significant physiological impairments, and withdrawal of food for a short interval without weight loss A precondition is that the diet must meet the full nutritional needs of the animals. **Examples** Addition of inert markers to diet Food withdrawal in adult salmonids for up to 24 hours
Catching and handling	---
Marking	---
Collection of samples	Removal of organs and tissues, including blood collection, after euthanasia **Examples** Collection of tissue samples for hormone or gene expression analysis Studies of functions in freshly isolated organs or cells
Toxicology	Application via water of pharmacologically inactive substances or active substances with known innocuous effects, if administered in a manner, quantity and frequency causing no stress for the animals **Examples** Vehicle control and reference groups to which no substances are administered
Genetically modified organisms	Maintenance and raising of morphants, mutants or genetically modified (transgenic) animals with no clinically detectable adverse phenotypes **Examples** Phenotypes with different pigmentation or stripe patterns that have no effect on social interaction with conspecifics

Behavioural biology	Non-invasive observation without disturbing the animal, with no previous treatment **Examples** Release experiments in bodies of water with physiological water quality Orientation in T-maze with rewarding of the correct choice by food
Infectiology and immunology	Asymptomatic infection with opportunistic micro-organisms or parasites

17.2 Fish in breeding facilities and laboratory animal facilities - Severity degree 1

Physical influences and husbandry	Manipulation of temperature, photoperiod, water chemistry or stocking density etc. such that a mild physiological disturbance may be expected during the adaptation period in fish and causing only a mild impairment of general condition **Examples** Changes in recommended stocking density for a limited time period
Feeding	Modified diets which are imbalanced or lack essential nutrients and are expected to cause metabolic changes outside the normal range, with mild clinically detectable impairments of general condition **Examples** Long-term administration of high-fat and low-protein diets
Catching and handling	Restriction of mobility for up to 24 hours, manipulations causing short-term mild pain or impairment of general condition **Examples** Catching with a net, trap or net bag Very brief manipulation out of water Transfer between containers with very short exposure to air Tight restraint for up to 1 hour Repeated or frequent weighing and measuring Catching by means of anaesthesia; immobilisation Egg collection by stripping anaesthetised female fish, without exposure to air Non-invasive imaging procedures with appropriate sedation or anaesthesia Intramuscular or intraperitoneal administration of substances in volumes appropriate to the size and species of animal Exposure of conscious fish to air for longer than “transfer from one water container to another”

Marking	Marking that causes little to no strain or marking that causes short-term mild pain or impairment of general condition **Examples** Marking by means of non-toxic and non-aversive dyes in the water Tagging of anaesthetised fish with a filament in the distal portion of the tail fin Application of external telemetry devices causing only minor impairment of normal behaviour Insertion of a telemetry device into the stomach by oral gavage under general anaesthesia, where the device does not have a significant effect on physiological function Dye marking behind the eye under anaesthesia Tail clipping for marking/DNA sampling
Collection of samples	**Examples** Repeated blood samples (total <10% of circulating volume) Blood sampling under anaesthesia. Volume and techniques depend on age, size and species. Anaesthesia and manipulation with exposure to air Removal of a small number of scales from anaesthetised fish for genotyping or age determination purposes Egg collection by stripping anaesthetised female fish, without exposure to air
Surgery	Interventions on the animal under general anaesthesia with negligible or short-term mild post-intervention pain, suffering or impairment of general condition. **Examples** Euthanasia of the animal at the end of the intervention while still under anaesthesia (preterminal experiment) Subcutaneous transplantations of organs without physiological function in the recipient animals Infliction of minor injuries causing mild transient impairment, e.g. minor mucous membrane abrasions or skin lesions Gastric lavage under anaesthesia
Genetically modified organisms	Maintenance and raising of morphants, mutants or genetically modified (transgenic) animals causing mildly impaired phenotypes **Examples** Mutations causing mild disturbances of normal behaviour. Genetic constructs producing GFP expression under promoter control and affecting social interaction with conspecifics

	Genetically modified animals with alterations in blood lipids Mutations affecting vision
Behavioural biology	Behavioural studies with stimuli which are associated with mild pain, suffering or anxiety and which the animals cannot immediately avoid. **Examples** Induction of stimuli briefly associated with negligible pain or suffering, which the animal can immediately successfully avoid (e.g. artificial predator with possibility of escape) Optokinetic response test Behavioural studies with positive reinforcement Passive avoidance test Conditioning with bitter-tasting food Short-term exposure to an artificial predator where immediate escape to a refuge is not possible Deprivation of social partners or short-term solitary housing of gregarious species Testing of substance efficacy in the open-field test Models with frequent alteration of environmental conditions, such as circadian rhythm models
Toxicology	Application of test substances in non-lethal doses followed by euthanasia of the animals or termination of the experiment in the event of mild clinical symptoms **Examples** Determination of drug-receptor binding *ex vivo* Determination of tissue levels by radiography Application of substances causing transient mild reactions, local or systemic, with no additional strain owing to the mode of administration or sample collection routes Performance of pELS tests in the early (pre-embryonic) life stage, causing mild impairment at later stages
Disease models	Models causing clinically manifest short-term mild impairment in the animal and models where the animals are euthanased on the onset of clinically detectable changes.
Infectiology and immunology	Infections or immune responses where only short-term, mild clinical symptoms or impairments of general condition are expected. **Examples** Ectoparasites or endoparasites in host fish, causing mild or transient irritation

	Application of inactivated parasites, bacteria or viruses (or constituents thereof) expected to cause only low-grade local inflammatory reactions; no repeated exposure or treatment Local graft-versus-host reactions Irradiation or chemotherapy resulting in immune-incompetence of limited duration with subsequent spontaneous reconstitution
Cancer	Tumour models causing no or short-term mild impairment of general condition, where the termination criteria exclude severe or chronic strain and where no cytostatics or other pharmacologically active substances are administered simultaneously. **Examples** Transplantation of cancer cells into fish for the purpose of passage of tumours

17.3 Fish in breeding facilities and laboratory animal facilities - Severity degree 2

Physical influences and husbandry	Manipulation of temperature, photoperiod, water chemistry or stocking density etc. such that moderate physiological disturbances may be expected during the adaptation period in fish or causing long-lasting mild disturbances with a low chance of adaptation
Feeding	Modified diets which are imbalanced or lack essential nutrients and are expected to cause metabolic changes outside the normal range, with moderate clinically detectable impairments of general condition which persist after the experimental period
Catching and handling	Manipulations causing transient moderate or chronic mild pain, suffering or anxiety or impairment of general condition without significant restriction of mobility Restriction of mobility for up to 1 week **Examples** Removal of fish from water for up to 1 minute Non-invasive imaging procedures associated with repeated sedation or anaesthesia and removal from water
Marking	Marking which causes transient moderate or chronic mild pain, suffering or anxiety or impairment of general condition **Examples** Attachment of external telemetry devices interfering with normal activity or behaviour Intramuscular or intraperitoneal implantation of telemetry devices by means of surgical procedures under anaesthesia
Collection of samples	Collection of body fluids in large quantities, in large numbers or at short intervals, or where sample collection is associated with prolonged application of measures causing strain, with or without previous administration of pharmacologically active

	substances (no toxic doses, no other severe interventions, no prolonged restraining measures applied previously) **Examples** Frequent blood sampling or collection of volumes greater than physiologically tolerated (>10% of body volume) Urine collection by insertion of a catheter into the bladder and attachment with suture material around the cloaca under anaesthesia
Surgery	Interventions on the animal under general anaesthesia with short-term moderate or longer-term mild post-intervention pain, suffering or anxiety, short- to medium-term severe impairment of movement/swimming/behaviour or food intake **Examples** Induction of minor wounds or skin lesions Removal of several scales Amputation of fin portions Subcutaneous transplantations of internal organs without physiological function in the recipient animal
Genetically modified organisms	Maintenance and raising of morphants, mutants or genetically modified (transgenic) animals causing moderately impaired phenotypes
Behavioural biology	Behavioural studies with stimuli associated with moderate pain, suffering or anxiety and which the animals can successfully avoid, or associated with mild strain which the animals cannot avoid **Examples** Stress models where habituation (adaptation) of the animal to the stimulus occurs, chronic stress through overstimulation Permanent illumination for a prolonged period (up to two weeks) Deprivation of social partners or solitary housing of gregarious species over an extended period Observation of behaviour under the influence of pharmacologically active substances expected to cause a complex modification in behaviour and a mild change in physiology Observation of behaviour under the influence of drugs such as ethanol or serotonin uptake inhibitors
Toxicology	Application of test substances in non-lethal doses followed by euthanasia of the animals or termination of the experiment in the event of moderate clinical symptoms **Examples** Application of substances or doses which are expected to cause severe reactions but where the animals are euthanased on the onset of clinically detectable changes

	Bioaccumulation tests in fish Dose-finding studies Chronic toxicity/carcinogenicity tests with administration of the test substance via the feed or water Reproductive toxicity Intraperitoneal injection of substances that can cause peritonitis Performance of pELS tests where the concentrations used in the early (pre-embryonic) life stage cause moderate impairment at later stages
Disease models	Models causing clinically manifest short-term moderate or longer-term mild impairment in the animal **Examples** Endocrine models such as hypophysectomy with hormone substitution Alloxan-induced diabetes with insulin treatment Senescence models with persistent mild or short-term moderate impairment of general condition
Infectiology and immunology	Infections or immune responses where short-term moderate or longer-term mild clinical symptoms or impairment of general condition are expected **Examples** Application of vaccines for tolerance studies, where the termination criteria exclude severe or prolonged strain Systemic infections of adult fish Models with induced endotoxin shock under anaesthesia, followed immediately by euthanasia Models causing an inflammatory reaction Experiments inducing local tissue reactions with transient disturbances of body function or general condition Irradiation or chemotherapy in sublethal or lethal doses with reconstitution of the immune system
Cancer	Tumour models causing short-term moderate or long-term mild impairment of general condition; cytostatics or other pharmacologically active substances may be administered simultaneously **Examples** Tumour growth models Transplantation of cancer cells into fish for the purpose of studying the development of metastasis Testing of substances with cytostatic properties

17.4 Fish in breeding facilities and laboratory animal facilities - Severity degree 3

Physical influences and husbandry	Manipulation of temperature, photoperiod, water chemistry or stocking density etc. such that severe physiological disturbances may be expected during the adaptation period in fish or causing long-lasting moderate disturbances with a low chance of adaptation **Examples** Saltwater/freshwater challenge outside of normal species-appropriate ranges
Feeding	Modified diets which are imbalanced or lack essential nutrients and are expected to cause metabolic changes outside the normal range, with severe clinically detectable impairments of general condition which persist after the experimental period.
Catching and handling	Manipulations causing transient severe or chronic moderate pain or impairment of general condition with or without significant restriction of mobility Restriction of mobility over an extended period (more than 1 week) **Examples** Removal of fish from water for up to 2 minutes
Marking	Methods of marking fish that are associated with mortality or significant interference with normal behaviour **Examples** Certain kinds of jaw tags Intraabdominal implantation of active transponders
Collection of samples	Collection of body fluids in relatively large quantities, in large numbers or at short intervals, or where sample collection is associated with prolonged application of measures causing strain, with or without previous administration of pharmacologically active substances, with previous application of toxic doses, other severe interventions or prolonged restraining measures
Surgery	Interventions on the animal under general anaesthesia with short-term severe or chronic moderate post-intervention pain, suffering, anxiety or impairment of general condition **Examples** Intestinal resection Cardiac resection Transplantations of organs with physiological function in the recipient animal
Genetically modified organisms	Maintenance and raising of morphants, mutants or genetically modified (transgenic) animals causing severely impaired phenotypes

Behavioural biology	Behavioural studies with stimuli associated with severe pain, suffering or anxiety which the animals can successfully avoid, or associated with moderate strain where the stimuli are repeatedly applied and the animals cannot avoid them **Examples** Pain studies in adult fish Models with chronic, frequently changing, moderate stress, with no adaptation occurring Experimental stress models where stressing events occur over a prolonged period of several weeks without any rhythm recognisable to the animal Observation of behaviour under the influence of pharmacologically active substances expected to cause a complex modification in behaviour and a moderate to severe change in general condition Forced swimming test with exhaustion as the endpoint
Toxicology	Application of test substances in lethal or sublethal doses **Examples** Toxicological studies expected to cause severe reactions and mortality Death as the endpoint Acute toxicity studies Performance of pELS tests where the concentrations used *in vitro* in the early (pre-embryonic) life stage are expected to cause severe impairment at later stages
Disease models	Models causing clinically manifest short-term severe or longer-term moderate impairment in the animal, without suitable treatment **Examples** Models giving rise to long-term moderate endocrine disturbances in the animal (decompensation) without suitable hormone substitution Senescence models with a long-term moderate or short-term severe impairment of general condition Studies of diseases that are known to be fatal and whose fatal course cannot be avoided Models with induction of clinically manifest severe cardiac insufficiency
Infectiology and immunology	Infections or immune responses where short-term severe or longer-term moderate clinical symptoms or impairments of general condition are expected **Examples** Chronic infections of adult fish with chlamydia or tuberculosis, where the termination criteria exclude severe or prolonged strain

	Application of vaccines for tolerance studies, where the termination criteria exclude severe or prolonged strain Infections characterised by progressive disease with lethal outcome Models of bacterial infections for screening of new antibiotics Models with induced endotoxin shock in animals allowed to awaken after anaesthesia, or in conscious animals Application of inactivated parasites, bacteria or viruses (or constituents thereof) expected to cause substantial inflammatory reactions, with subsequent repeated exposure to test the immune response (immunogenicity tests) or subsequent treatment with other substances Vaccine efficacy testing Generalised graft-versus-host reactions Experiments inducing local tissue reactions with disturbances of body function or general condition Experiments inducing generalised inflammatory reactions such as autoimmune reactions Mercury-induced glomerulonephritis Any immunisation of animals with endogenous tissue which gives rise to an autoimmune disease if the experiment is not terminated prematurely Irradiation or chemotherapy with a lethal dose without reconstitution of the immune system, or with reconstitution resulting in the production of graft-versus-host disease
Cancer	Tumour models causing progressive lethal disease associated with chronic moderate pain, suffering or impairment of general condition; cytostatics or other pharmacologically active substances may be administered simultaneously **Examples** Slow-progressing cancer without premature termination Therapeutic dose models Tumour therapy models Tumours causing cachexia Invasive bone tumours Models with ulcerating and necrotising tumors

E Legislation

***Tierschutzgesetz* (Animal Protection Act, AWA) of 16 December 2005 (SR 455)**

As at 1 May 2017

Art. 4 AWA	Principles
Art. 16 AWA	Painful surgical procedures
Art. 17 AWA	Limitation to the indispensable minimum
Art. 19 AWA	Requirements

***Tierschutzverordnung* (Animal Protection Ordinance, AWO) of 23 April 2008 (SR 455.1)** As at 20 March 2018

Art. 137 AWO	Criteria for assessing the essential measure of animal experiments that entail strain on the animal
Art. 140 AWO	Conditions for approval of animal experiments

Ordinance of the FSVO on laboratory animal husbandry, the production of genetically modified animals and the methods of animal experimentation (*Tierversuchsverordnung* (Animal Experimentation Ordinance), AEO; SR 455.163) of 12 April 2010

As at 1 May 2010

Art. 24 AEO	Categories of strain resulting from experimental procedures or measures
Art. 25 AEO	Categories of strain due to genetic modifications

FEDERAL FOOD SAFETY AND VETERINARY OFFICE