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Guidelines*/** to help evaluate the stress factor for laboratory animals (tab. 1.6.7) during authorized animal experiments

(Effective September 21st 2010)

Anatomic structures and neurophysiologic mechanisms for pain perception are equal in both humans and animals. It is thus legitimate to assume that a stimulus that

- causes pain in humans,
- destroys or potentially destroys living tissue or
- causes avoidance reactions

will produce an experience of pain in animals, too.

The Principle (§ 1) of the German Animal Welfare Act of May 25th 1998 reads: "The aim of this Act is to protect the lives and well-being of animals, based on the responsibility of human beings for their fellow creatures. No-one may cause an animal pain, suffering, or harm without good reason."

The act allows one exception to this principle in § 7, stating that experiments on vertebrates can be carried out only under the premise that the expected pain, suffering, or harm inflicted on the laboratory animal is ethically justifiable in view of the purpose of the experiment. The experimenter is required to face that issue and is expected to assess the potential stress the laboratory animal might be exposed to while being tested.

The following proposals are provided in an effort to help the experimenter assess the suffering, harm, or pain his study might cause an animal in a prospective, realistic manner. They presuppose that the performed study procedure is state-of-the-art. They are not meant to supersede a responsible stress evaluation through the individual investigator. In addition to this prospective evaluation, it is indispensable for the laboratory animal to be monitored closely, as individual animals, also depending on their species, might react differently to what was expected before the study began. If several procedures are combined, the stress level for the animal will most likely increase. Also the duration of an experiment can affect the stress level significantly. The presented table is provided to help not only with a prospective evaluation but also with the search for experimental designs that are less stressful for the animal.

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* Referring to Moyal, Zur Belastung von Tieren im Tierversuch, Diss. Hannover 1999, and the Swiss Federal Veterinary Office, Einteilung von Tierversuchen nach Schweregraden vor Versuchsbeginn, 1995

** Compiled with the aid of colleagues from Bergholz-Rehbrücke and Rostock.

Preliminary note: ***Classification of stress levels, always in consideration of time***

No stress	Mild stress	Moderate stress	Severe stress
No impact	Procedures or operations performed on animals for experimental purpose that cause **mild, short-term stress** (pain, harm) short-term: up to 24 hours maximum	Procedures or operations performed on animals for experimental purpose that cause **moderate, short-term, or mild, medium- to long-term stress** (pain, suffering, or harm, fear, impaired general condition) medium-term: up to 14 days maximum	Procedures or operations performed on animals for experimental purpose that cause **severe to extremely severe, or moderate, medium- to long-term stress** (severe pain, continuous suffering, severe fear, or severely impaired general condition)

The time scale given above is a rough guideline and can vary, depending on the animal species.

Indicators to objectify the severity of experimentally induced diseases (suffering, pain, harm)*

	Mild stress	Moderate stress	Severe stress
Weight reduction (compared to the strain specific weight)	mild (< 5%)	5 – 20%	> 20%
Condition of fur, grooming, stomata, catarrhal phenomena	Temporary oculonasal discharge; no self mutilation	Scruffy, shaggy fur; permanent oculonasal discharge; no self mutilation	Scruffy, shaggy fur with dehydration (skin fold); permanent, severe oculonasal discharge; self mutilation
Deviation of temperature from physiological temperature	Up to 1°C		More than 2°C
Breathing, heart rate	Change of HR < 30%; normal breathing	Temporarily abnormal breathing	Change of HR >30%, increased for longer periods; heavy breathing
Spontaneous behaviour, motor function, posture, sleeping posture, social behaviour	Social interaction with cage companions; no convulsions	Minor interaction with cage companions; intermittent crouching posture; quivering; convulsions	The animal is isolating itself; the animal is tiptoeing or showing a heavy gait; permanent crouching posture, quivering, convulsions.
Reaction to artificial stimulation; catching behaviour; reaction to physical contact	The animal reacts normally to stimulation.	Attention and behavioural reaction is diminished or exaggerated.	Not responsive

* Modified according to Morton DB, Griffith PHM (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and hypothesis for assessment. Vet Rec 116:431-436 and FELASA (1994)

Stress table, article 1:

***Application and aspiration without producing diseases* (attachment 1.6.7, application for approval)**

	No stress	Mild stress	Moderate stress	Severe stress
Application		Substance applications with short-term compulsory measures and not combined with other procedures. The substance may inflict only mild stress on the animal. With multiple applications, time must be taken into consideration! Oral treatment (except gavage tubes for large animals) Local applications: - at the eye, on the skin, on the mucosa Injections: - intradermal, intramuscular, intravenous - intraperitoneal - subcutaneous (transponder as well) **Under anaesthesia only:** -,intracerebral - intubation	Substance applications with short-term compulsory measures and not combined with other procedures. The substance may inflict only moderate stress on the animal. With multiple applications, time must be taken into consideration! Oral treatment of large animals with use of gavage tubes. **Under anaesthesia only:** - intracardial, orbital sinus	Substance applications with severe restrictions on motion and not combined with other procedures. With multiple applications, time must be taken into consideration!
Aspiration		Sampling of body fluids in small quantities and small numbers with only short-term compulsory measures and not combined with other procedures - venipuncture - Foley catheter for larger animals **Under anaesthesia only:** - final puncture of the heart	Sampling of body fluids in small quantities and small numbers with only short-term compulsory measures and not combined with other procedures **Under anaesthesia only:** - venipuncture with indwelling catheter - bladder aspiration - gallbladder aspiration - bone marrow aspiration - peritoneal lavage - retrobulbar (repeated puncture of the same eye after a fortnight latency) - indwelling cannula in the cisterna magna	Sampling of body fluids with severe restrictions on motion and not combined with other procedures

Stress table, article 2: *Infection studies* (attachment 1.6.7, application for approval)

		Mild stress	Moderate stress	Severe stress
	The duration of infections is often a crucial stress factor!	Short-term infections with mild clinical symptoms (e.g. local abscess).	Short-term infections with moderate, or chronic infections with mild clinical symptoms (e.g. diarrhoea).	Progressive infections with lethal consequence, or chronic infections with distinct clinical symptoms (e.g. paralysis).
Bacteria		Induction of localised bacterial dermatitides with several pathogens If combined with pruritus or hyperaesthesia → moderate stress	- Implantation of a tissue chamber, later being completely colonized with bacteria. - RITARD model (removable intestinal tie adult rabbit diarrhoea model) with enterotoxic Escherichia coli	- Models with induction of bacterial synovitis, e.g. with Borrelia burgdorferi (Lyme disease), in immunosuppressed animals - CASP[1] model for inducing a septic clinical picture - LPS[2] injections in inflammation models
Viruses		Spumavirus or immunodeficiency virus in cats	- Induction of a human influenza B infection in mice - infections with hepatites non-A, non-B in non-human primates	- Intracerebral infection of mice with LCM virus (lymphocytic choriomeningitis) - rat model for herpesvirus encephalitis
Parasites		Subclinical infections in immunocompetent animals with pathogens of parasitic colon diseases (giardia, coccidia trichostrongyles, hookworms)	Infections with pathogenic doses of trichostrongyles, lung worms, tissue parasites (e.g. metacestodes of Echinococcus, **but only initially**; in later stages, depending on the location, severe stress is possible) and blood protozoa (trypanosomes, babesia). Principally, low to medium infestations with ectoparasites (e.g. ticks, fleas, or flies) can be rated as moderate.	Infections with high doses of trichostrongyles, lung worms, tissue parasites (e.g. metacestodes of Echinococcus), blood protozoa (trypanosomes, plasmodia, babesia), or ectoparasites (e.g. mange mites)
Fungi		Induction of geotrichoses, without pruritus only	Application of low pathogenic fungi, like Rhodotorula rubra or Candida glabrata on the back of a guinea pig with diabetes	Induction of a pulmonary aspergillosis (allergic reactions caused by aspergillosis spores)

[1] Colon Ascendens Stent Peritonitis, [2] Lipopolysaccharides are no longer rated as infectious agents, the pathogenic effect, however, of e.g. enterobacteriaceae among others is based on this molecule. The effects can range from induction of mild sepsis (5 mg/kg) up to an anaphylactic shock (15 mg/kg) .

Stress table, article 3: *Surgical procedures under anaesthesia without recovering* (attachment 1.6.7, application for approval). Those procedures are done under general anaesthesia with the animal being killed at the end of the experiment while still being anaesthetized. This is considered to cause **mild** stress.

Stress table, article 4: *Other surgical procedures* (attachment 1.6.7, application for approval)

	No stress	Mild stress	Moderate stress	Severe stress
		Smaller surgical and other procedures on animals (minor tissue traumas) under general or local anaesthesia with mild postsurgical pain, suffering, and mildly impaired general condition	Surgical and other procedures on animals under general anaesthesia with moderate postsurgical pain, suffering, or moderately impaired general condition	Surgical and other procedures on animals under general anaesthesia with severe postsurgical pain, suffering, or severely impaired general condition over longer periods of time
4.1 Abdominal cavity / chest cavity		- Mini-pump s.c. or i.p. - subcutaneously applied central venous catheter - skin biopsies - application of cannulas in peripheral blood vessels - orchidectomy without laparotomy - generation of skin papillomas - subcutaneous transplantation of organs without physiological function in the receiving animal - subcutaneous implantation of tumour tissue, subject to tumour size - invasive blood pressure measurement under anaesthesia - rumen fistulas in cattle and small ruminants	<u>Models with laparotomy:</u> ovariectomy, vasectomy, adrenalectomy, hepatectomy, hysterectomy, Caesarean section, lymphadenectomy, thyroidectomy, partial nephrectomy, splenectomy. - bowel resection, depending on location and extent - implantation of catheters into the Aorta abdominalis or the bile duct - mini-pumps i.v. - mini-pumps with substance release into the stomach - generation of a stomach fistula - small bowel fistula in pigs and ruminants	- Models with thoracotomy - nephrectomy (>50%) - bowel resection, depending on location and extent - stomach resection - transplantation of a functional inner organ

Continuation stress table, article 4: *Other surgical procedures* (attachment 1.6.7, application for approval)

4.2 Musculoskeletal system			Application of implants in the intact musculoskeletal system	- Joint transplantations - application of implants in the musculoskeletal system, followed by functional loss of that system
4.3 CNS / sensory organs (eye, nose ear)			- Implantation of indwelling catheters in cerebral ventricles or of electrodes in the cerebric, without restrictions on motion - hypophysectomy with hormone substitution	- Trauma studies - models that cause severe, clinically manifest endocrine disorders, e.g. hypophysectomy without hormone substitution
4.4 Others e.g. tumour implantation e.g. invasive blood pressure measurement e.g. procedures in peripheral tissue			- Aortic banding - localised tumours - cornea transplantation - transplantation of organs without physical function in the receiving animal (except subcutaneous localisation) - models with skin transplantations, without severe restrictions on motion	- Tumours with metastases leading to tumour cachexia or other lethal diseases - transplantation of organs with physiological functions in the receiving animal (when failure of which would effect severe stress) - traumatically induced circulatory shock - application of cannulas into functional end arteries

Stress table, article 5: *Physical impacts* (attachment 1.6.7, application for approval)

	No stress	Mild stress	Moderate stress	Severe stress
5.1 Radiance	Diagnostics (Dexa, scintigraphy, X-ray)	- Depending on the dose	- Depending on the dose - reversible tissue damage	- Depending on the dose - irreversible tissue damage
5.2 Electric power	- Physiological area (60 – 90 mV) - ergotherapy (different animal species often with different sensitivity level)	- Electrophysiological measurements under anaesthesia - avoidance behaviour with given alternative to avoid the situation, depending on voltage, amperage, and duration (e.g. pasture fence device, 9 V, short-term)	- No alternative to avoid the situation, depending on voltage, amperage, and duration - reversible tissue damage	- No alternative to avoid the situation with higher voltage and amperage, and longer duration - irreversible tissue damage
5.3 Traumatization		**Under anaesthesia:** - trepanation of the skull **Under final anaesthesia:** - Surgical techniques - replacement tissue - Bone fracture, wound healing - closed traumas of the muscular tissue		**With recovering from anaesthesia:** - Bone fracture, wound healing - closed traumas of the muscular tissue without analgesia (Sudeck's disease models) - surgical techniques, replacement tissue - generating and subsequent treating of traumatic brain injuries
5.4 Burns	Reaction test hot plate. The purpose of this test is to provoke a reaction, not to induce pain.	Burns or scalds first-, second-, third-degree in defined areas. Maximum 10% of the body surface of a pig for simulated treatment of severely burnt humans, done under anaesthesia only and with final artificial coma.	Burns or scalds first-, second-, third-degree in defined areas. Maximum 30% of the body surface of a pig for simulated treatment of severely burnt humans, done under anaesthesia only and with final artificial coma.	Burns or scalds first-, second-, third-degree in defined areas. Maximum 50% of the body surface of a pig for simulated treatment of severely burnt humans, done under anaesthesia only and with final artificial coma.
5.5 Others (acids/bases) Pressure change Sound Magnetic fields	Depending on - concentration and type - quantity - route of application - duration Natural air pressure Depending on the decibel level (up to 70db) Diagnostics (NMR)	→→→ Pressure change, depending on time and area of impact Depending on the decibel level (up to 85db)	→→→ →→→ Depending on the decibel level (from 85db on)	→→→ →→→ Depending on the decibel level (from 100db on)

Stress table, article 6: *Generation of pain* (attachment 1.6.7, application for approval)

	No stress	**Mild stress**	**Moderate stress**	**Severe stress**
		Experiments that cause short-term mild pain: - Hot plate test - tail flick test without restrainer - tail immersion test - writhing test with 0,25ml diluted suspension of phenyl-p-benzoquinone 0,02% in tragacanth 0,4%	Experiments that cause short-term moderate pain, or chronic mild pain, without considerable restrictions on motion: - All models with acute paw oedema with parameter "withdrawal" - all models with acute paw oedema with parameter "paw volume", duration of experiment < 6 hours - tail flick test with restrainer - writhing test with < 0,2ml of 2% acetic acid, or with 0,4ml of 1% diluted acetic acid - writhing test with alcoholic solution of phenyl-p-benzoquinone 0,02% in tragacanth 0,4%	Experiments that cause short-term severe pain, or chronic moderate to severe pain, with or without considerable restrictions on motion: - All models with acute paw oedema with paramater "vocalization" - all models with acute paw oedema, duration of experiment > 6 hours - application of noxious stimuli, without means of escape or avoidance behaviour - writhing test with > 0,2ml and > 2% of diluted acetic acid - causing of anatomical and physiological damage, combined with stress or distress

Stress table, article 7: *Toxicity studies* (attachment 1.6.7, application for approval)

It is basically an exceptional case when laboratory animals in toxicity tests are exposed to mild stress, since the OECD guidelines principally ask for the highest tested dose (of usually 2-3 dosage groups) to cause clear toxic effects.

	No stress	**Mild stress**	**Moderate stress**	**Severe stress**
7. Toxicity studies		- Compatibility studies in which animals, due to type of application or sampling, are exposed to only mild stress, and only temporary, mild local or systemic reactions are to be expected - procedures under lethal anaesthesia - short-term compulsory measures, combined with few pain/distress	- Compatibility studies in which animals, due to type of application or sampling, are exposed to no severe stress, and permanent, moderate local or systemic reactions are to be expected; lethality is not expected - procedures under anaesthesia with recovering from anaesthesia and minor subsequent damage (analgesia not necessary) - short-term compulsory measures, combined with considerable pain/distress - permanent compulsory measures (e.g. jacket for infusion pump), combined with few stress	- Compatibility studies in which severe pathophysiological states are induced, and fatalities are to be expected - procedures under anaesthesia with recovering from anaesthesia, in which subsequent analgesia is necessary or longer convalescence periods are needed -
7.1 Acute (e.g. OECD 402-406, 420, 423, 425, 429)		- Acute toxicity tests, local compatibility tests, and sensitisation tests, limited to the induction phase (e.g. LLNA), and without use of adjuvant, in which only mild pathophysiological reactions and no fatalities are to be expected	- Acute toxicity tests and local compatibility tests, in which moderate pathophysiological reactions and no fatalities are to be expected - Sensitisation tests with application of adjuvant, or challenge (e.g. maximization test, optimisation test), in which moderate pathophysiological reactions are to be expected	- Acute toxicity tests in which severe pathophysiological states or fatalities are to be expected - local compatibility tests and sensitisation tests, in which severe local or systemic reactions are to be expected

Continuation stress table, article 7: *Toxicity studies* (attachment 1.6.7, application for approval)

	No stress	Mild stress	Moderate stress	Severe stress
7.2 Subacute (e.g. OECD 407-413, 424)		- Subacute and subchronic toxicity tests with repeated application up to 3 months, where no or only mild pathophysiological reactions and no fatalities are to be expected	- Subacute and subchronic toxicity tests with repeated application up to 3 months, where moderate pathophysiological reactions and no fatalities are to be expected	- Subacute and subchronic toxicity tests with repeated application up to 3 months, where severe pathophysiological reactions and fatalities are to be expected
7.3 Chronic (e.g. OECD 451-453)		- Chronic toxicity tests / cancerogenity tests with application of the test substance in food or drinking water, in which no or only mild pathophysiological reactions and no fatalities are to be expected	- Chronic toxicity tests / cancerogenity tests with oral application of the test substance, in which moderate pathophysiological reactions and no fatalities are to be expected - parenteral, dermal, and inhalative application with minor pain or stress exposure	- Chronic toxicity tests / cancerogenity tests, in which the application of the test substance is combined with considerable pain or stress exposure, or in which severe pathophysiological reactions or fatalities are to be expected
7.4 Reproduction toxicology (e.g. OECD 414-416, 421-422)		- Reproduction toxicology tests in one or several generations, where no or only minor maternal toxicity is to be expected, and the surviving offspring is expected to experience no to little impact - prenatal development toxicity tests (teratogenity tests) in which no or only mild pathophysiological reactions and no fatalities are to be expected	- Reproduction toxicology tests in one or several generations, where moderate maternal toxicity is to be expected (meaning an effect exceeding that of a slight decrease in body weight gain), or where the surviving offspring is expected to experience moderate impact - prenatal development toxicitiy tests (teratogenity tests) in which moderate pathophysiological reactions and no fatalities are to be expected	- Reproduction toxicology tests in one or several generations, where considerable maternal toxicity is to be expected, and the surviving offspring is expected to experience considerable impact - prenatal development toxicitiy tests (teratogenity tests) in which severe pathophysiological reactions and fatalities are to be expected

Stress table, article 8: *Behavioural impairment* (attachment 1.6.7, application for approval)

	No stress / no ae	**Mild stress**	**Moderate stress**	**Severe stress**
8.1 Aversive learning	Positive conditioning	Models with short-term mild pain, suffering, or fears; ability for the animal to successfully avoid the situation	Models with short-term moderate pain, suffering, or fears; ability for the animal to successfully avoid the situation	Models with severe pain, suffering, or fears; inability for the animal to avoid the situation
8.2 Deprivations	**With two factors combined, the stress level is upgraded automatically.**			
8.2.1 Social		Increased population density, avoidance behaviour without consequence, restrictions on social behaviour, no complete isolation	Overpopulation, fight-induced damage, complete isolation of social animals up to 1 week	Overpopulation, death caused by stress, complete isolation of social animals from 1 week on
8.2.2 Sleep		Changing of illumination phases with slightly extended dark phases and short-term disruption of sleep behaviour	Changing of illumination phases with moderately extended dark phases and medium-term disruption of sleep behaviour	Changing of illumination phases with highly extended dark phases
8.2.3 Water		Deprivation up to 12 hours	Deprivation 12-24 hours	Deprivation more than 24 hours
8.2.4 Food	Deprivation: less than 2 nutrition cycles	Deprivation: 2 nutrition cycles	Deprivation: 3-4 nutrition cycles	Deprivation: more than 4 nutrition cycles
8.2.5 Motion	According to husbandry acts	Below the requirements of husbandry acts, up to 24 hours	Below the requirements of husbandry acts, more than 24 hours and up to 7 days maximum	Considerably below the requirements of husbandry acts, up to 4 weeks maximum
8.3 Overstimulation		- Stress leading to alarm reaction - short-term stress up to 24 hours	- Stress leading to resistance phase, chronic stress - models without adapting to the stressor	- Stress leading to exhaustion phase - models with chronic, often changing, potent stressors
8.4 Others	Testing of husbandry types in accordance with the regulations, and with the customary	All markings when catching is done state-of-the-art (sender up to 5% of the body weight)		

Stress table, article 9: *Other procedures/treatments* (attachment 1.6.7, application for approval)

	No stress	Mild stress	Moderate stress	Severe stress
Generating states of disease				
- Metabolism			- Diabetes with treatment	- Diabetes without treatment
- Inflammation		- Anti-pyrexia in rats with LPS (0,1 mg/kg) or IL-1 - arachidonic acid test on the mouse ear	- Air pouch model in rats - encephalomyelitis model in which animals are killed during the first exacerbation - screening of anti-inflammatory drugs in mouse strains with spontaneously occurring immune mediated disease	- Pertussis pleurisy in mice/rats - recurrent encephalomyelitis model without killing the animals during the first exacerbation - Sudeck's disease models
- Arthritis			- Adjuvant arthritis with killing of the animals < 19 after generating the arthritis (killing at first sign of the characteristic of arthritis) - collagen II induced arthritis with early killing of the animals	- Adjuvant arthritis when duration of experiment >18 days after generating the arthritis - carragheen arthritis model - induction of arthritis in inbreeding mice with borrelia spirochetes
Tumour induction		Models with subcutaneously localised tumours, where no functional disorders are caused in the animal, and no cytostatic drug is administered	Models not leading to tumour cachexia or other progressively lethal diseases, or being cancelled before functional disorders occur in the animal	Models leading to tumour cachexia or other progressively lethal diseases, or not being cancelled before functional disorders occur in the animal
Genetically modified animals*	Phenotype does not differ from wild type	Phenotype with mild disorders and mild clinical pictures, e.g. change in blood lipids	Phenotype with moderate disorders and moderate clinical pictures, e.g. increase in aggression, anxiety	Phenotype with severe disorders and severe clinical pictures, e.g. haemophilia

* Since apart from the desired changes there can also be side-effects, the phenotype, with every line, is to be assessed comprehensively.

Continuation stress table, article 9: *Other procedures/treatments* (attachment 1.6.7, application for approval)

	No stress	**Mild stress**	**Moderate stress**	**Severe stress**
Immunisation	Not existing	Without irreparable tissue damage: subcutaneous, intramuscular, intravenous for DNA, proteins	- Intraperitoneal with Freund's adjuvant or generation of ascites, without considerable increase in volume - - intramuscular with subsequent necrosis, depending on size and location (Depending on the extent, it can also be rated as severe.)	- Pads - intraperitoneal with generation of ascites and increase in volume - intramuscular with subsequent necrosis, depending on size and location
Metabolic cages	Stress depends on different factors (e.g. ambient temperature, spatial restrictions with and without fixation, social contact, ground configuration, duration) and is varying significantly from species to species. The severity of the experienced stress needs to be assessed individually with regards to the governing factors and the respective animal species, and cannot be listed here in tabular form. What applies for every animal species: The better conditioned the animals are, the less stressful it is for them to be tested or the longer the tests can take. With animals lacking a sense of time, every fixation is a severely stressful experience for them, when they are not used to it (positive learning). As husbandry in farming is not humane, it cannot be rated as a relevant guideline (sow farrowing crates, tie-stalls).			