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Options for assessing constraints in animal experiments

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Contents

Definitions

- Constraint
  Sum of all factors that have a direct or indirect impact on anatomical, physiological, cognitive or emotional states and result in temporary or lasting pain or suffering.
- Prospective constraint assessment
  Definition of overall constraint before the start of the experiment, which results cumulatively from the assessment of all individual constraints to be expected in the course of the study.
- Current constraint assessment
  Assessment of the constraints for individual animals at the current point of time in the study.
- Retrospective constraint assessment
  Evaluation of overall constraint of the individual animal over the entire course of the study based on the results of the current assessments of constraints collated at the end of the study.
- Withdrawal from study
  Immediate suspension of the ongoing study for the animal concerned. This may also mean euthanasia of the animal.

Preliminary remarks

An important aspect in the planning of an animal experiment is the most realistic possible estimation of the related constraints to which animals are likely to be exposed in the course of the research project. The prospective constraint assessment serves to minimize the experimental constraints for laboratory animals as required by law (e.g. § 7 Para. 1, No. 2, § 8 Para. 1, No. 7 TierSchG, § 17 TierSchVersV). Those responsible for planning experiments, as well as the leads and deputy leads of research projects, are required to formulate sound prospective constraint assessments. At the same time, criteria must be defined that are as clear and easy to record as possible, allow the persons conducting the experiment and the animal care staff to recognize current constraints on individual animals at any time during the course of the experiment, record them and, if necessary, take appropriate action to reduce the constraints.

Prospective constraint assessment

Besides any pain to be expected, experiment-related constraints also include all other physical impairments and all psychological restrictions that reduce the wellbeing of the animals. The assessment of psychological constraints in laboratory animals poses a particular challenge. Comprehensive knowledge of the species-specific biology of the animal species used in terms of anatomy and physiological characteristics and of their species-specific behaviour is therefore an indispensable prerequisite for the necessary expertise of anyone who designs an experiment, as well as the study lead and deputy lead.

The prospective constraint assessment must additionally cover all periods of time during which laboratory animals are used for the purpose of the experiment. This also includes experimental phases in which, for example, the animals are given fixed amounts of food or certain diets in order to establish physiological baseline conditions that are as standardized as possible at the start of the actual experiment or to induce disease models (e.g. diabetes, obesity, non-alcoholic steatohepatitis, etc.), or also changed housing conditions (e.g. solitary confinement, metabolic cage, food deprivation). If the animals are to live on after the end of the study, long-term consequences of the procedures and treatments conducted in the study must also be taken into account. Phenotypic changes resulting from genetically engineered modifications must also be included in the constraint assessment. Forms are available for a formalized constraint assessment of genetically engineered animals[1].

Catalogues that summarize the constraints of standard procedures in animal experiments, e.g. in Annex VIII of Directive 2010/63/EU, in which certain constraints are assigned to individual procedures by way of example, serve as a means of standardizing prospective constraint assessments. Since the list of procedures listed there cannot reflect the diversity of procedures and treatments actually carried out, the person designing the experiment must list the specific constraints for each research project and assess their severity. Based on the severity of the individual constraints and their frequency, an overall degree of severity (“mild”, “moderate” or “severe”) is assigned to the project, which corresponds at least to the highest individual degree of severity. Final experiments performed entirely under general anaesthesia from which animals do not recover consciousness are classified as “non-recovery” in accordance with Annex VIII of Directive 2010/63/EU.

The prospective constraint assessment occupies an important position in the approval and notification procedure for animal research projects. Applicants should therefore attach particular importance to its scope and presentation.

Constraint assessment criteria

In addition to general criteria that take into account the species-specific anatomy and physiology as well as species-specific behaviour, study-specific criteria are also considered for the constraint assessment. Both include quantitative as well as qualitative and temporal criteria. When it comes to recording changes not only in the behaviour or external appearance of an animal, but also in most physiological parameters, natural activity patterns must be taken into account. The selection of the criteria recorded must be based on the actual circumstances. A single constraint criterion, such as body weight, which is often used because it is simple and objective, cannot always be used as the sole assessment criterion, but must be considered in the context of other clinical examinations and assessment criteria.

Clinical parameters, such as pulse, blood pressure or respiratory rate, for example, can be measured directly and therefore appear to be particularly objective, but measuring them in the

[1] https://www.bfr.bund.de/cm/343/beurteilung-der-belastung-genetisch-veraenderter-maeuse-und-ratten- version-2.pdf
https://www.bfr.bund.de/cm/343/beurteilung-der-belastung-genetisch-veraenderter-knochenfische-teleostei.pdf

day-to-day routine has obvious limits when it comes to small laboratory animal species. The same applies to food and water intake, which cannot be reliably determined for individual animals in housed in groups and fed *ad libitum*. If general clinical parameters are to be measured solely for the purpose of constraint assessment, it is necessary to consider whether the additional constraint imposed by the measurements required for this purpose might not outweigh the benefit of the constraint assessment.

In animal research, as is customary in medicine, symptom-based scores can also serve to classify the current severity of a disease or constraint status. The symptoms suitable for a scoring system are model or study-specific symptoms

1. whose occurrence is to be **expected in principle**. They are classified according to an increasing degree of constraint. This classification can be selected independently of severity grades “mild”, “moderate” and “severe”,
2. which are **not generally expected**, but which may occur as study-related complications, e.g. wound infections, loosened implants and the like. These are also classified according to an increasing level of constraint.

The severity of symptoms and constraints classified in this way is associated with directions on the action to be taken. The aim of the action(s) is to reduce the constraint within a defined period of time. If a maximum level of constraint defined in the planning of the study is exceeded, despite all the treatment measures taken, this leads immediately to withdrawal from the study.

As stipulated in § 16 TierSchVersV, it is a basic prerequisite that all persons involved must have the necessary species-specific expertise in biology, behaviour and physiology to make a competent identification of species-specific symptoms or constraints and to classify them according to the corresponding constraint categories.

Score sheets

Formalized assessment sheets, i.e. score sheets, are often used to record and classify acute constraints. Score sheets can be useful in the assessment of constraints, but are not always the best or even the only means available and should therefore not be considered mandatory.

Score sheets are not useful in final experiments for which all procedures are performed under anaesthesia and the laboratory animal does not recover consciousness. If the anaesthesia is a state-of-the-art procedure, there is no constraint for the laboratory animal beyond the induction of anaesthesia. Accordingly, such procedures are assigned the severity grade “non-recovery” according to Annex VIII of EU Directive 2010/63/EU.

Score sheets are intended to ensure that constraints are classified objectively on the basis of defined criteria which can be applied equally by everyone involved in a project. With the aid of score sheets, acute constraints can thus be recorded and assessed continuously in a way that enables them to be tracked over time. This allows both a better prediction of whether, to what extent and over what period of time an increase in constraints is to be expected and also a reliable check on whether measures taken to reduce constraints have been successful. Score sheets also help to obtain a comprehensive picture of the cumulative overall constraints by

recording various criteria (Joint Working Group on Refinement 2011, Bugnon et al. 2016). Despite every effort to assess constraints objectively through the use of score sheets, individual differences in the assessment of qualitative criteria cannot be avoided when several observers are involved (Keubler et al. 2018).

Examples of score sheets can be found in a great many publicly accessible sources (e.g. Fentener van Vlissingen et al. 2015; Graf et al. 2016, Kanzler et al 2016, Lang et al. 2016; Martins et l. 2016, Palle et al. 2016; Pinkernell et al. 2016). To ensure that meaningful use can be made of these score sheets, they must be adapted in all cases to the research project concerned.

To simplify the use of the score sheet, it is advisable to adapt the constraint assessment criteria to the actual work processes in the animal facility. Criteria that are recorded from the observation of the undisturbed animal, such as general behaviour or external appearance of the animal, should be assessed first in the score sheet, followed by criteria that require increasing manipulation of the animal (Bugnon et al. 2016).

Many research projects cause changes in just one organ or cell system. In these cases, the general criteria of a score sheet are often not sensitive enough to serve as indicators of experiment-related constraints. So it is always necessary to include all the criteria that could be affected as a direct consequence of malfunctions in the altered organ or cell system.

The time intervals in which the acute constraints of the animals is checked must be shortened with increasing intensity of the constraints. However, this must not lead to additional, avoidable constraints due to increased controls, such as frequent weighing.

If a research project involves surgical procedures that are followed by a prolonged observation period, and if constraints resulting from the procedure only occur for a relatively short period of time, it makes sense to draw up and use different constraint criteria, observation intervals or score sheets for the different phases of the experiment. Both the criteria used to assess the constraint and the time intervals at which they are recorded should be adapted to the set-up of the experiment. The animals may need to be checked daily or multiple times after the surgical procedure to detect acute complications, but in the further course of the study these controls may cause the animals unnecessary stress without significantly improving the constraint assessment. Assessments by the animal care staff and veterinary supervisors can also serve as a supplement to the recording of current constraints using the score sheet.

Regardless of how many criteria are assessed and how detailed the assessment of individual criteria, a score sheet must provide a quick and unequivocal assessment of the constraint at all times. Instructions for action must also be formulated in a clear and easily understandable way and be easy to implement.

Different assessment systems are conceivable for recording the current severity of constraints with the aid of score sheets. For example, individual symptoms can be assigned numerical values that reflect the severity in a points system. The overall constraints are then derived from the sum of the various numerical values or, in individual cases, from predefined individual values. When certain threshold values are reached, measures defined in the score sheet are

implemented in order to reduce the constraint. These might include shortening the observation intervals, extending recovery phases, taking therapeutic measures and involving the animal welfare officer and/or the veterinarian responsible. However, to ensure that constraints can be realistically assessed using a points system and that appropriate treatment measures are initiated, it must be borne in mind that cumulative effects may result in the overall level of constraints being greater than the (calculated) sum of the individual constraints. As an alternative to the assessment based on points, constraint levels can also be determined by the occurrence of individual symptoms or combinations of symptom and identified e.g. by letters or other symbols. Conditions under which measures to reduce constraints are initiated may also be defined if no score sheet is used because depending on the study, for example, the recording of a single critical criterion is sufficient.

Instructions

The aim of constraint assessment and evaluation is to ensure that laboratory animals are not subjected to constraints beyond what is indispensable for the experiment and as far as possible to minimize the constraints on each individual laboratory animal in the experiment. So measures must also be defined that help to reduce acute constraints. In score sheets, this requirement is usually met by instructions aimed at reducing individual constraint criteria or the overall level of constraints when a certain severity or score is reached. These instructions are symptom-specific and must not influence the result of the experiment. If the severity of constraints is high, the affected animal may need to be shown to the veterinarian or animal welfare officer responsible, who may initiate further measures.

Humane endpoints

In the planning of an animal experiment, clear criteria for a suspension or discontinuation of the experiment in the individual animal, i.e. humane endpoints, must be defined. Before such criteria are applied, the constraints that actually occur must first be recorded and evaluated in order to reduce constraints, if necessary, through appropriate interventions such as additional analgesia, feeding with high-calorie diets, postoperative refinement measures or modifications of housing conditions. The occurrence of symptoms that prevent the objective of the experiment being achieved is also usually considered a humane endpoint.

If the ethically acceptable maximum overall level of constraints defined in the study design is exceeded for an individual laboratory animal, the experiment must be discontinued immediately in this animal. If suspension of the experiment cannot be expected to result in a decrease in the severity of the constraints, or if a suspension is not possible in view of the study design, the animal must be withdrawn from the experiment. The use of a score sheet is also not absolutely essential for assessing whether criteria for humane endpoints have been met. It is crucial that fulfilment of the withdrawal criterion is unequivocally recognized and appropriate action taken. If there is any ambiguity, the lead investigator should reach a decision with the animal welfare officer and/or the veterinarian responsible.

Retrospective constraint assessment

The aim of the retrospective constraint assessment is to record the constraints that actually occurred in the experiment for each individual laboratory animal and to compare them with the constraints expected on the basis of the prospective constraint assessment. The retrospective assessment allows the criteria used for the constraint assessment in the study to be reviewed. It is to be expected that, as a result of the retrospective constraint assessment, the assessment of constraints for subsequent research projects will be improved not only by the addition of additional criteria, but also by the omission of unsuitable criteria. This will also lead to a more realistic assessment of the severity of the constraint under which the project is classified. The retrospective constraint assessment of the individual animal also provides information on the constraints addressed in the annual laboratory animal report as stipulated in the German Regulation on Laboratory Animal Reporting (VersTierMeldV), so that different degrees of severity may have to be reported for animals within a given project.

The retrospective constraint assessment in the sense described here is not to be confused with the retrospective assessment of research projects according to §35 TierSchVersV, which must be carried out by the approval authority for certain research projects based on information provided in the approval decision.

Species-specific symptoms of constraints

Listed below are criteria for various animal species used in animal experiments that can be used for constraint assessment and evaluation. Criteria from these lists can also serve as a basis for drawing up score sheets, but must be supplemented by experiment-specific criteria. The specification of physiological data has been deliberately left out, because such variables cannot be recorded for many of the animal species used, or only with huge technical effort. However, if these variables are determined for experimental purposes, they provide additional criteria for the classification of constraints and should definitely be included. Body condition and the so-called painful face of many animal species can contribute decisively to constraint assessment and should be included whenever this would appear reasonable and possible. Where species-specific assessment tools for the body condition score and the grimace scale are available in the literature, their source is indicated in the following tables.

Constraint criteria for fish

External appearance	Skin	– Loss of pigment – Changes of colour – Injuries/damage to fins
	Body posture	– Body tilted to the side – Body tilted upwards or downwards
Behaviour	Spontaneous behaviour	– Passivity – Increased resting on the aquarium floor
	Social behaviour	– No response to attacks by conspecifics – Increased aggressiveness
	Motor activity	– Limited – Swimming behaviour changed – Repeatedly coming to the surface for air
Nutrition intake	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Tachypnoea (increased movement of the gill cover)
Body Condition Score	Zebrafish: Wilson et al. 2013, Clark et al. 2018	

Constraint criteria for African clawed frog

External appearance	Skin	– Loss of pigment – Bleeding (ecchymosis) – Detachment of mucous layer
	Body	– Increased circumference (e.g. balloon disease)
	Body posture	– Body tilted to the side or – Body tilted upwards or downwards
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Hiding away
	Motor activity	– Limited – Swimming behaviour changed – Repeatedly coming to the surface for air
Nutrition intake	Food intake	– Reduced – Refused

Constraint criteria for reptiles

External appearance	Skin	– Shell necrosis (turtles) – Discoloration not appropriate to the situation (e.g. persistent blackening of the throat area in bearded dragons) – Permanent, severe obliteration of the femoral pores (bearded dragons)
	Eyes	– Sunken eyes
	Anogenital region	– Prolapse of the hemipenes (bearded dragons) – Cloacal prolapse
	Body posture	– Reduced muscle tone
Behaviour	Spontaneous behaviour	– Apathy – Dystocia or immature eggs laid as a result of stress – No reaction of extremities or head on manipulation (turtles)
	Motor activity	– Limited – Swimming at an angle or staying permanently on land (turtles)
Nutrition intake	Water intake	– Reduced – Refused (dehydration)
	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Breathing noises – Open-mouth breathing – Raised head and upper body (bearded dragon)

Constraint criteria for birds

External appearance	Plumage	– Dull – Ruffled – Dirty – Loss of feathers outside moulting season
	Cloaca	– Dirty – Prolapse
	Body posture	– Hunched back – Kinked tail – Titling of the head – Drooping wings – Reduced muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Feather ruffling / picking – Cannibalism – Reduced curiosity – Abnormally trusting – Stereotypical movements – Shift in natural activity phases / altered circadian rhythm
	Motor activity	– Limited – Ataxia – Inability to fly – Inability to sit on the perch
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Reduced vocalization
Nutrition intake	Water intake	– Reduced – Refused (dehydration) – Polydipsia
	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Open-mouth breathing – Breathing noises – Tail bobbing synchronized with breathing – Buccal breathing (overinflation of the infraorbital sinus)

Constraint criteria for mice

External appearance	Fur and skin	– Dull – Ruffled up (piloerection) – Dirty – Hair loss
	Eyes	– Sticky – Clouded – Closed/half-closed – Discharge
	Nose	– Discharge
	Abdomen	– Circumference increased – Sunken
	Anogenital region	– Dirty – Discharge – Rectal prolapse – Vaginal prolapse – Penis prolapse
	Body posture	– Hunched back – Head permanently tilted – Cowering posture – Reduced muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Change in nest-building – Self-mutilation – Stereotypies – Shift in natural activity phases / altered circadian rhythm
	Motor activity	– Stilt/tip-toe walking – Scuttling – Stiff gait – Tremor – Lameness – Paralysis – Convulsions
	Social behaviour	– Reduced – Isolation – Aggressiveness – Change in nest-building
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive

Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Shallow – Laboured breathing – Gasping
Body Condition Score	Ullman-Culleré MH & Foltz CJ 1999	
Mouse Grimace Scale	Langford et al. 2010	

Constraint criteria for rats

External appearance	Fur and skin	– Dull – Ruffled up (piloerection) – Dirty – Hair loss
	Eyes	– Sticky – Clouded – Closed/half-closed – Discharge – “Spectacle eyes” (secretion of Harderian gland)
	Nose	– Discharge
	Abdomen	– Circumference increased – Sunken
	Anogenital region	– Dirty – Diarrhoea – Discharge – Rectal prolapse – Vaginal prolapse
	Body posture	– Hunched back – Tucking or tensing of abdomen – Head hanging down – Cowering posture – Legs tucked under the body – Reduced muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Self-mutilation – Reduced exploration / curiosity – Stereotypies – Teeth grinding – Whimpering – Shift in natural activity phases / altered circadian rhythm – Disturbed sleep-wake rhythm
	Motor activity	– Stilt/tip-toe walking – Scuttling – Stiff gait – Tremor – Lameness – Paralysis – Convulsions
	Social behaviour	– Reduced

		– Isolation – Aggressiveness
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Vocalization when touched – Aggressiveness
	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused – Allotriophagy (pica behaviour)
	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Shallow – Laboured breathing – Breathing noises – Gasping
Body Condition Score	Hickman & Swan 2010	
Rat Grimace Scale	Sotocinal et al. 2011	

Constraint criteria for neonatal mice and rats

External appearance	Skin	– Pallor (anaemia) – Grey skin colour – Dehydration
	Development	– Retarded compared with littermates – Malformations (e.g. hydrocephalus, limbs)
	Body posture	– Stretched lying position
Behaviour	Spontaneous behaviour	– Isolation from rest of litter – Righting reflex reduced
	Motor activity	– Reduced wriggling activity
	Maternal care	– Neglect – Cannibalism
Nutrition intake	Milk intake	– Reduced – Milk spot absent

Constraint criteria for hamsters (golden hamsters and short-tailed dwarf hamsters)

External appearance	Fur	– Dull – Ruffled up (piloerection) – Dirty – Hair loss
	Eyes	– Sticky – Clouded – Bulbous – Discharge
	Nose	– Discharge
	Cheek pouches	– Continuously filled – Only one side filled
	Abdomen	– Circumference increased
	Scent glands	– Clogged flank glands in the golden hamster – Clogged abdominal glands in the dwarf hamster
	Anogenital region	– Dirty – Diarrhoea – Discharge – Rectal prolapse – Vaginal prolapse
	Body posture	– Hunched back – Head hanging down – Cowering posture – Legs tucked under the body – Reduced muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Stereotypies – Self-mutilation – Teeth grinding – Whimpering – Shift in natural activity phases / altered circadian rhythm – Increased recumbent periods during darkness – Resting outside the burrow dug by the hamster itself

	Motor activity	– Scuttling – Stiff gait – Tremor – Lameness – Paralysis – Convulsions
	Social behaviour	– Unusual aggression
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Vocalization when touched – No retraction of the front paws when setting up – Lack of aggression when disturbed in the resting phase
Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Shallow – Laboured breathing – Breathing noises – Gasping

Constraint criteria for guinea pigs

External appearance	Fur and skin	– Dull – Ruffled up (piloerection) – Dirty – Hair loss
	Eyes	– Sticky – Clouded – Closed/half-closed – Sunken – Discharge
	Nose	– Discharge
	Abdomen	– Circumference increased – Sunken
	Anogenital region	– Dirty – Changed skin colour – Diarrhoea – Discharge
	Body posture	– Hunched back – Head hanging down – Cowering posture – Reduced muscle tone – Relief posture / weight shifting between limbs
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Self-mutilation – Teeth grinding – Chirruping/twittering – Soft squeaking – High-pitched squealing – Hypersalivation – Sounds of pain when urinating – Disturbed sleep-wake rhythm
	Motor activity	– Tip-toe walking – Scuttling – Stiff gait – Tremor – Absent rotation of head, absent rotation of body – Lameness – Paralysis – Convulsions

	Social behaviour	– Unusual aggression
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive
Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Increased respiratory rate (especially rapid, shallow breathing) – Reduced respiratory rate – Laboured breathing – Breathing noises – Gasping
Body Condition Score	https://www.pfma.org.uk/_assets/docs/pet-size-o-meter/pet-size-o-meter-guinea-pig.pdf Ara et al. 2012	

Constraint criteria for rabbits

External appearance	Fur and skin	– Dull – Ruffled up (piloerection) – Dirty – Hair loss
	Eyes	– Sticky – Clouded – Discharge
	Nose	– Discharge
	Abdomen	– Circumference increased – Sunken – Taut
	Anogenital region	– Dirty – Diarrhoea – Discharge
	Body posture	– Hunched back – Belly tucked up – Head hanging down – Cowering posture – Pressing of belly on the floor – Reduced muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Self-mutilation – Teeth grinding – Cries of pain (only acute) – Hypersalivation
	Motor activity	– Stiff gait – Absent hopping – Tremor – Lameness – Paralysis – Convulsions
	Social behaviour	– Reduced – Isolation – Marked aggression
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Aggressiveness – Increased timidness – Sharp cries of pain

Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused – Allotriophagy (pica behaviour)
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Laboured breathing – Breathing noises – Open-mouth breathing
Body Condition Score	Sweet et al. 2013 Prebble et al. 2015 http://www.pfma.org.uk/_assets/weigh-in-wednesday/pet-size-o-meter-rabbit.pdf	
Rabbit Grimace Scale	Keating et al. 2012 Hampshire and Robertson 2015	

Constraint criteria for dogs

External appearance	Fur and skin	– Dull – Ruffled up (piloerection) – Dirty – Hair loss
	Eyes	– Sticky – Clouded – Sunken – Bulbous – Discharge – Colour of conjunctiva
	Nose	– Discharge – Crusting
	Abdomen	– Circumference increased – Tucked up / taut – Sunken
	Anogenital region	– Dirty – Diarrhoea – Discharge
	Body posture	– Hunched back – Head hanging down – Relieving posture – Tail tucked in – Reduced or tightened muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Intensive licking of body parts – Self-mutilation – Stereotypies – Unusual vocalization sounds
	Motor activity	– Stiff gait – Tremor – Lameness – Convulsions
	Social behaviour	– Reduced – Isolation – Aggressiveness
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Increased timidness – Aggressiveness – Defensiveness or vocalization

Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused – Vomiting
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Increased costal or abdominal breathing – Laboured breathing – Breathing noises
Body Condition Score	https://www.aaha.org/globalassets/02-guidelines/nutritional-assessment/nutritionalassessmentguidelines.pdf Laflamme 1997 Witzel et al. 2014	

Constraint criteria for sheep

External appearance	Wool	– Dirty – Dull – Loss of wool
	Eyes	– Sticky – Clouded – Discharge
	Nose	– Discharge
	Abdomen	– Circumference increased – Sunken (paralumbar fossa)
	Anogenital region	– Dirty – Diarrhoea – Discharge
	Body posture	– Hunched back – Cramped posture
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Teeth grinding – Looking round or kicking legs after touching of body part – Rumination reduced or absent – Restlessness – Frequent standing up and lying down
	Motor activity	– Stiff gait – Tremor – Lameness – Convulsions
	Social behaviour	– Reduced – Isolation – Seeks protection of the group
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Acute crying out

Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test)
	Food intake	– Reduced – Refused
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Increased costal or abdominal breathing – Laboured breathing – Breathing noises
Body Condition Score	Russell (1984) Phythian et al. 2012 https://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex9622/$FILE/bcs-sheep.pdf	
Sheep Grimace Scale	Guesgen et al. 2016 Häger et al. 2017	

Constraint criteria for pigs

External appearance	Skin colour	– Pale (greyish) – Blueish (cyanotic) – Hyperaemic
	Hair coat	– Dirty – Ruffled
	Eyes	– Sticky – Clouded – Sunken – Discharge
	Nose	– Discharge
	Abdomen	– Circumference increased – Tucked up
	Anogenital region	– Dirty – Diarrhoea – Discharge
	Body posture	– Hunched back – Scuttling – Cramped posture – Legs tucked under the body when lying – Increased lying phases
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Teeth grinding – Hiding (withdrawal behaviour) – Restlessness – Aggression
	Motor activity	– Stiff gait – Tremor – Lameness – Convulsions
	Social behaviour	– Reduced – Isolation
	Reactions to external stimuli and manipulation	– Slowed down – Hyperactive – Increased timidness – Increased flight reactions

Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused – Vomiting
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Increased costal or abdominal breathing – Laboured breathing – Open-mouth breathing – Breathing noises
Body Condition Score	http://www.thepigsite.com/articles/275/assessing-sow-body-condition/ Muirhead & Alexander 1997	
Pig Grimace Scale	Di Giminiani et al. 2016 Viscardi et al. 2017	

Constraint criteria for non-human primates

External appearance	Coat	– Dull – Ruffled (piloerection)
	Eyes	– Sticky – Clouded, glass – Discharge – Prolapsed nictitating membrane (especially in marmosets)
	Nose	– Discharge
	Anogenital region	– Dirty – Diarrhoea – Discharge
	Body posture	– Crouching – Arm folded across chest – Reduced muscle tone
Behaviour	Spontaneous behaviour	– Passivity – Apathy – Reduced self-grooming – Hyperactivity – Self-mutilation – Clenching lips or teeth together – Increased chewing – Teeth grinding – Stereotypies – Shift in natural activity phases / altered circadian rhythm
	Motor activity	– Stiff gait – Tremor – Lameness – Convulsions
	Social behaviour	– Reduced – Reduced self-grooming – Isolation – Changes in temperament (e.g. sudden aggression in an animal that is essentially friendly)

	Reactions to external stimuli and manipulation	– Slowed down – Interest in new ambient stimuli absent or markedly reduced – Hyperactive – Increased or reduced vocalization – Increased timidness – Increased flight reactions
Nutrition intake	Water intake	– Reduced – Refused (dehydration; skin fold test) – Polydipsia (polyuria)
	Food intake	– Reduced – Refused – Hyperphagia – Vomiting
Breathing	Dyspnoea	– Increased respiratory rate – Reduced respiratory rate – Increased costal or abdominal breathing – Laboured breathing – Breathing noises
Body Condition Score	Clingerman & Summers (2012) Summers et al. (2012)	

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