

# The reporting of clinical signs in laboratory animals

FELASA Working Group Report

JM Fentener van Vlissingen<sup>1</sup>, M Borrens<sup>2</sup>, A Girod<sup>3</sup>, P Lelovas<sup>4</sup>,  
F Morrison<sup>5</sup> and Y Saavedra Torres<sup>6</sup>

Laboratory Animals  
2015, Vol. 49[4] 267–283  
© The Author(s) 2015  
Reprints and permissions:  
sagepub.co.uk/  
journalsPermissions.nav  
DOI: 10.1177/0023677215584249  
la.sagepub.com



## Abstract

Observing and reporting clinical signs in laboratory animals is necessary for many reasons: the assessment of animal welfare, compliance with the principle of refinement (e.g. humane endpoints), regulatory compliance (e.g. reporting severity) and, importantly, as a scientific outcome, e.g. in animal models of disease or safety studies. Developments in the reporting of clinical signs will enhance the scientific value gained from animal experiments and further address the ethical cost. This paper discusses systematic approaches to the observation and reporting of clinical signs in animals (to be) used for research. Glossaries from public and corporate institutions have been consulted and a reference glossary has been set up, providing terminology to be tailored for institutional or project-specific use. The clinical examination of animals must be carried out by competent and specifically trained staff in a systematic way and repeated at adequate intervals and clinical observations must be registered effectively to allow this information to be used. The development of institutional or project-specific glossaries and the use of handwritten records or automated databases are discussed in detail. Among the users are animal care staff, veterinarians and researchers who will need to agree on a given set of clinical signs to be monitored routinely or as a scientific read-out and to train for the proper application. The paper introduces a long list of clinical signs with scientific terminology, descriptions and explanations as a reference glossary to be published and maintained online as a living document supported by the authors as an editorial committee.

## Keywords

animals, clinical signs, glossary, humane endpoint, refinement

Clinical signs are objective terms for describing abnormalities that are detected during a clinical observation or examination of an animal.

The reporting of clinical signs in laboratory animals is important for a number of reasons:

1. *Retrospective reporting.* In certain European countries, for example in The Netherlands, the gathering of such information serves to fulfil the requirements of retrospective reporting on severity. The Federation of European Laboratory Animal Science Associations (FELASA) supports the prospect of adopting retrospective reporting of scientific procedures on animals across Europe<sup>1</sup> and this issue is currently implemented as part of the new European Directive 2010/63.<sup>2</sup>

<sup>1</sup>NVP (Nederlandse Vereniging voor Proefdierkunde), Erasmus Laboratory Animal Science Center EDC, Erasmus MC, Rotterdam, The Netherlands

<sup>2</sup>BCLAS (Belgian Council for Laboratory Animal Science), Rommel Consulting Partners, Lab Animal Sciences and Medicine, Jodoigne, Belgium

<sup>3</sup>AFSTAL (l'Association Française des Sciences et Techniques de l'Animal de Laboratoire), Insourcing Solutions (IS) CRF, Charles River, L'Arbresle, France

<sup>4</sup>HSBLAS (Hellenic Society of Biomedical and Laboratory Animal Science), Center for Experimental Surgery, Biomedical Research Foundation, Academy of Athens, Athens, Greece

<sup>5</sup>LASA (Laboratory Animal Science Association, UK), Imperial College, Faculty of Medicine, South Kensington Campus, UK

<sup>6</sup>SECAL (Sociedad Española para las Ciencias del Animal de Laboratorio), MRC National Institute for Medical Research, The Ridgeway, Mill Hill, London, UK

## Corresponding author:

J M Fentener van Vlissingen, Erasmus Laboratory Animal Science Center EDC, Erasmus MC, Rotterdam, The Netherlands Erasmus MC, PO Box 2040, 3000 CA Rotterdam, The Netherlands.  
Email: m.fentener@erasmusmc.nl

2. *Assessment of animal welfare.* Data on clinical signs are instrumental for the recognition of pain, disease and distress in animals. Both specific and non-specific clinical signs can indicate severe discomfort to animals.<sup>3</sup> Carstens and Moberg<sup>4</sup> reviewed guidelines for the recognition and assessment of pain and distress in laboratory animals and published a table of indicators of pain that can be detected from the general behaviour, appearance and basic physiology of the animal.
3. *Effective communication* between animal care staff, technicians, attending veterinarians and investigators. Clinical signs indicative of a health or welfare concern can be communicated objectively and consistently.
4. *Humane endpoints.* Clinical signs are critical for the definition and application of the principles of humane endpoints. Ideal humane endpoints are those that can be used to end studies before the onset of pain and distress.<sup>5</sup> There are many excellent references on the use of clinical signs to define humane endpoints.<sup>6–13</sup>
5. *Phenotyping.* Reporting of clinical signs observed in animal models such as genetically-altered animals allows for improvements in phenotyping. This reporting should include the area of scientific interest, which is valuable for further characterizing the model, as well as those areas that may affect animal welfare.<sup>14</sup> Such information is essential for addressing the ethical cost–benefit analysis of the generation or use of such animal models. The endorsed Working Document on genetically-altered animals<sup>15</sup> drafted in the course of the implementation of Directive 2010/63 provides specific regulations and guidance on this.
6. *Safety/efficacy studies* in pharmacology and toxicology. Reporting of clinical signs is routinely done in this field and aids the interpretation of clinical pathology in such studies.
7. *Scientific outcomes.* Accurate data capturing should include any scheduled observations, as well as unscheduled observations, made during the *in vivo* phase of the research. This regards the phenotyping of models or any other scientific outcomes of research. These data will also contribute to serendipity, as unscheduled observations will be acquired systematically and may throw light on hypotheses under study or lead to novel ones. Also, unscheduled observations allow for timely expanding of specific observations, sample collection and analyses needed to complete the picture.
8. *Scientific publication.* There is a general lack of reporting of the clinical signs seen in animal models in scientific publications, except when the clinical signs are an outcome parameter. The collection of standardized clinical data from laboratory animals may improve the quality of the scientific

data processed by academics and may encourage the inclusion of such information in scientific publications. This, in turn, would potentially enhance animal welfare by providing input for project review and the refinement of procedures and models.

Historically in the laboratory animal field, clinical signs have been observed and recorded in toxicological research as part of the identification of dose-dependent adverse effects. Technicians responsible for the daily care of the animals carry out the observations and registrations. To this end, glossaries have been developed to standardize descriptive terminology.<sup>16</sup> Glossaries enable the data collected to be more accurate, favour descriptive terms, and avoid diagnostic terms (e.g. ‘distended abdomen’ is preferable to ‘pregnant’).

Many toxicology laboratories use automated systems to register observations on clinical signs as a part of the study outcomes. The use of standardized glossaries and automated systems is not as yet customary in most pharmaceutical companies or in academia but is clearly gaining ground.

The FELASA Working Group on ‘The reporting of clinical signs in laboratory animals’ was established to propose a glossary of clinical signs, after review of both public and privately owned (corporate) documents, that can model for a system for systematically observing and reporting clinical signs in animals used in research. The glossary would comprise a list of terms with definitions and background information that is scientifically precise and suitable for reference use by animal caretakers, technicians and investigators. The terms would only describe observations that can be made without the use of clinical instrumentation (e.g. a thermometer or weighing scales). The Working Group has chosen to restrict this task to mammalian laboratory species, with a particular bias to rodents.

This report aims to discuss the considerations relating to the reporting of clinical signs, which involves technical support, training and means of registration. The method of collection needs to be standardized in order to standardize the collection of data. This would include a method of examining the animal and examples of adaptable score sheets for recording observations.

The Working Group is mindful of the added bureaucratic burden that reporting of clinical signs will bring to an animal unit and will present the views of those in the field who are already practising such reporting.

## Clinical observation and examination

### Daily inspection

Daily inspection is to be done by persons who are familiar with the species and particularities that

individual animals may have within normal limits. The room should be sufficiently lighted during observation. The observations should adhere to the following protocol:

1. When entering the animal room, be observant of any abnormal sounds (or lack of usual background noise), smells and other conditions. Check any notes on the management of the room or the animals.
2. Observe the cages for any abnormal deposits, substrate disruption (or lack of maintenance of the nesting material by the animals), feed and drinking water (both supply and gradual consumption over time, in case of *ad libitum* administration, or leftovers, in case of meal feeding), etc.
3. Observe each group for general activity level and any abnormal behaviour such as an animal dissociated from the group.
4. Next, observe the animals one by one. Observe each animal from a distance for any abnormal behaviour, body contour, coat changes, etc.
5. Next, when approaching the cage or pen, observe the response to external stimuli. In case the cage needs to be handled to view individual animals, as may be necessary when they are housed in containment such as an individually-ventilated cage (IVC) or an isolator, the response to stimuli is an integrated part of the initial observation (steps 2–4).

For daily inspection, the animals need not be handled, except where prior procedures or the observation of clinical signs require close attention. Only animals that need closer observation should be identified by reading their permanent individual mark (ear notch, tattoo, toe mark, transponder, or similar mark) or a temporary mark which can be applied to identify a single animal when group housed (for which there are many options, e.g. coat or skin coloration using a felt pen).

### *Clinical examination*

The identification of the animal is read.

Whenever animals need to be handled, for cage changes or procedures, they should be examined more closely. For this, the identification of the animal should be read and a simplified scheme for general clinical examination should be followed (Table 1).

It is important to note whether a condition is generalized, symmetric or localized. Standardized anatomical nomenclature should be used to refer to specific parts or areas of the body (Figure 1).

The behavioural response to capturing/handling should be noted, in particular abnormal passiveness

or abnormal adverse reactions. Special conditions, such as aggressive animals or biocontainment conditions may prohibit the handling of animals for clinical examination. In such cases, the scheme should be followed by observation only, which may require more time than in a daily inspection and may be limited regarding some of the observations that can be made easily during handling (e.g. the ventral side of the body). When the animal is in hand, coat and skin texture and general muscle tone should be noted, as well as any behavioural responses such as withdrawal reactions. Observations such as skin temperature or moisture may be obstructed by wearing gloves but personal protective equipment must not be disregarded for routine clinical examination.

The scheme for clinical examination may appear elaborate but can be completed quickly by an experienced person. Only the definition and recording of a sign upon first observation will take some extra time.

Clinical examination may need to be repeated more frequently in a more focused way in the follow-up to a procedure or following up on the prior observation of a clinical sign until the conditions become stable or ameliorate.

The description of a clinical sign consists of the description of an abnormality, and its location, using anatomical terms. Anatomical terminology used by veterinarians can be very detailed. For common use, a simplified approach is preferred, as is outlined in Figure 1. It is important to indicate whether an abnormality occurs only on one side (left or right) or symmetrically. Reporting can be helped by using a simple graphic to indicate the location and size of a lesion, but this will mostly be done in a model- or species-specific setting.

The description of an abnormality should be given using terminology from a predefined glossary. Then, qualifiers can be added to indicate the size, extent (such as the amount of discharge), as well as chronicity (acute or sudden, subacute, chronic or permanent) which is reported as a repeated observation. Furthermore, day-to-day changes can be reported (increasing, decreasing, or healing). In the absence of a suitable predefined term, veterinary experts should be asked to provide one.

The reporting of clinical signs must not be confused with the reporting of diagnoses. A diagnosis is a conclusion to be made from clinical and additional diagnostic observations or measurements (e.g. blood analysis, microbiological testing). A clinical sign indicates some sort of abnormal condition but not its cause. An analysis of the cause will start with a differential diagnosis listing possible causes, taking into account their plausibility, in part based on other concomitant signs, the experimental use of the animal and

**Table 1.** Clinical examination.

Area of interest	Parts considered	Detailed observation and reporting	Instruction
General	Oversight	Development related to age and sex. Nutritional state, quality of the hair coat. Behavioural features and locomotor abnormalities.	Observe animal in home cage/pen and in response to stimuli; next, handle it as needed.
Head	Head (total)	Observe for shape, relative size of parts and symmetry, hair cover, position before you focus on details below.	You need to see both sides and the view from the front.
	Nasal orifices	Opening, colour, moisture, any discharge, any asymmetry, any respiratory sounds.	View from the front, do not handle without reason, compare with normal aspect for the species.
	Mouth (lips, cheek, teeth, gums, tongue, cheek pouches)	Position, texture, colour, moisture, any wounds or deformities, abnormal discharge.	The lips may be lifted manually but the jaws need not be opened. Note any other features such as smell. Cheek pouches (dependent on species) may extend to the side of the neck and be empty or filled (e.g. with food).
	Eyes, eyelids	Position, size of the eyeball, position and close fit of the eyelids. Moisture, reflection, transparency of cornea, size and shape of pupil, any discolorations or discharge.	Be familiar with species-specific features such as pigmentation of the iris and form of the pupil, as well as reflection of light within the eye, in some species. Abnormalities may occur unilaterally or symmetrically (note).
	Ear (pinna, ear canal)	Shape, thickness, position of the external ear (pinna), inspection of the ear canal, any abnormal content or discharge.	There is a lot of variation between species and even within species (e.g. different breeds of dogs, pigs, rabbits), thus, the observer must be familiar with the normal presentation. Inspect for symmetry, ear movements, inspect the ear canal without or with handling of the pinna.
Neck	Part between head and shoulders	Mobility, shape, any wounds or masses, any guttural sounds.	Mobility is checked by observing the animal moving its head. The dorsal side consists of vertebral column, ligaments and muscles. The skin is loose in most species and then the preferred site to check on dehydration by observing the sliding back of the skin after picking up a skinfold. The ventral side contains a lot of different organs (lymph nodes, salivary glands, oesophagus, larynx, trachea, thyroid gland) that warrant a careful inspection and sometimes gentle palpation.
Trunk	Back	Position, symmetry, muscles.	The back has a thoracic part connected to the ribs, and a lumbar part where ribs are absent, connecting the thoracic part to the pelvis. The relative length and flexibility of the lumbar part varies between species. The position of the back (flexion up or down, or sideways) must be noted. The

(continued)

**Table 1.** Continued

Area of interest	Parts considered	Detailed observation and reporting	Instruction
	Thorax	Shape, respiratory movements.	<p>visibility of the spine depends on the amount of musculature and subcutaneous fat and is indicative of the nutritional state of the animal.</p> <p>The frontal part of the rib cage is covered by the shoulders that are part of the trunk but technically the upper (most proximal) part of the front limbs. The area between the thorax and the front limb is the axilla ('armpit'). The thorax contains the heart and the lungs. Behind the shoulders, the ribs are superficial and allow for observation of the shape of the chest and respiratory movements. In some species, mammary glands are present at the ventral and lateral aspects of the thorax.</p>
	Abdomen	Shape, tension, superficial structures, (respiratory) movements.	<p>The abdomen contains many vital organs such as the gastrointestinal tract, liver, spleen, and urogenital organs. Internally, it is separated from the thorax by the diaphragm which extends forward. Seen from the side, the lungs extend backwards between the diaphragm and the ribs. Parts of the abdomen are only covered by skin and muscles, in the flanks (lumbar area, caudal to the ribs and ventral to the lumbar spine) and ventrally (between the sternum and the pelvis). The shape and tension can be evaluated in these areas. The abdominal wall moves with respiration and sometimes (movements of) abdominal organs can be seen. Superficial structures are the mammary glands and the preputial opening in males (species-dependent).</p>
	Pelvis	<p>Shape and symmetry of skeleton and muscles of the pelvis.</p> <p>Structures of the perineum: size, shape, colour, any discharge.</p> <p>Structures of the groin, with special attention to mammary glands in this area, and inguinal lymph nodes.</p>	<p>The pelvis is the bony structure that connects the hindlimbs to the trunk. The outside is covered by large muscles of the limb and is best observed from the back, the side or from behind. Inside is a canal where the rectum and urogenital organs pass backwards to end at the perineum, where muscles and skin contain their openings. Between the hindlimb and abdomen lies the inguinal area (the groin) where major blood vessels pass and – sometimes prominent – inguinal lymph nodes are located. The structures are best observed from behind or from the ventral side and their arrangement differs between species and sexes.</p>
Limbs	Front limbs	Position, motility, any wounds or deformities, abnormal muscle	<p>The upper part (the shoulder) is part of the trunk. Although the general design is conserved across species, there are major</p>

(continued)

**Table 1.** Continued

Area of interest	Parts considered	Detailed observation and reporting	Instruction
		tone or muscle mass, proper use (versus lameness).	adaptations to the use (locomotion: flight, walking on toe or sole of the foot; other use such as holding objects). The observer needs to be familiar with species-specific features. To indicate the site of a lesion, use proximal (closer to the trunk) or distal (towards the end of the extremity), medial (inside) or lateral (outside), anterior (side of the head) or posterior (side towards the tail), and for the foot dorsal for the upper side and palmar for the side towards the ground.
	Hindlimbs	Position, motility, any wounds or deformities, abnormal muscle tone or muscle mass, proper use (versus lameness).	Although the general design is conserved across species, there are major adaptations to the use (walking on toe or sole of the foot). The observer needs to be familiar with species-specific features. To indicate the site of the lesion, terminology is the same as for the front limb, except the underside of the foot that is referred to as plantar.
Tail		Position, motility, tension (laxness or rigidity), any wounds or deformities.	The skeleton of the tail is the most caudal part of the spine and is rudimentary in some species. However, many species have a longer tail which can be moved by muscles and long tendons. The skin can be sparsely haired or covered with fur or plume, dependent on the species. Handle the tail to detect abnormal tension.

epidemiological considerations. Veterinary textbooks<sup>17</sup> and other handbooks (such as Pritchett-Corning et al.<sup>18</sup>) will show typical examples of conditions. Also, users of glossaries of clinical signs may have reference pictures showing typical examples of clinical signs listed. Such pictures are very useful as references supporting the description of clinical signs. However, to make a diagnosis from a picture alone would be inadequate and can often lead to false conclusions.

## Users

### *Animal care staff*

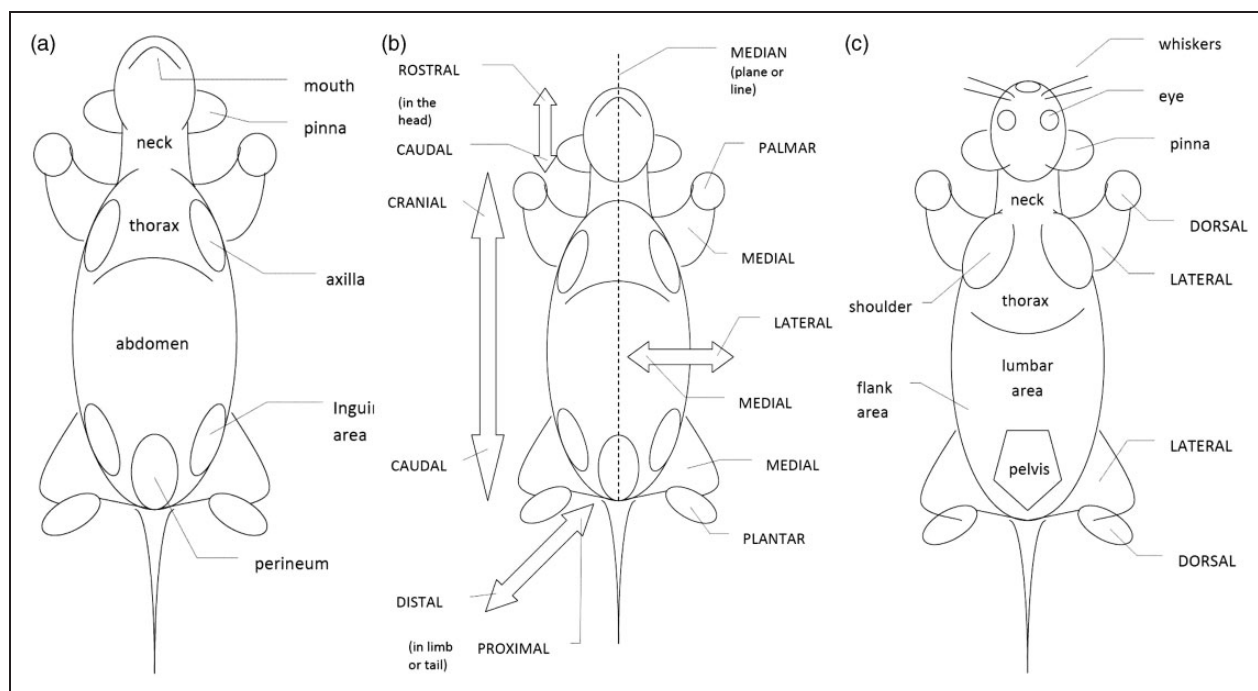
Animal care staff should be competent (educated and trained) to work with the type of animals in their care and to recognize normal and abnormal animal behaviours and external features. For breeding situations, this competence should include an understanding of normal reproduction and development. For animal research models, relevant information on the

model should be readily available and consulted when needed.

Animal care staff should be competent to relate clinical signs to (affected) well-being, ill health, pain, and/or distress; and to report on this and to act on such observations in a responsible way. These reports should become more informative and objective by applying systematic approaches to observation and examination of animals and the use of standard terminology. Secondly, the reporting of clinical signs allows for better follow up (e.g. by a colleague on weekend duty or by consulting the veterinarian) and incorporation of these observations into the scientific outcome of the study. Animal care staff should use the reporting system in place and, if this is not a notification system, notify any senior staff, veterinarian and/or investigator depending on the local organizational structure and procedures.

Education for these functions should cover the biology (anatomy, physiology, ethology) of the species as well as health and welfare management, and breeding





**Figure 1.** Simple graphics indicating clinical terminology to map clinical signs onto the animal: (a) Ventral aspect of an animal indicating different parts of the body. (b) Ventral aspect of an animal indicating directional terms relative to the position in the body. (c) Dorsal aspect of an animal indicating different parts of the body (in lower case) directional terms relative to the position in the body (in upper case).

management as appropriate. Staff should be explicitly trained in the observation and examination of animals, as well as the use of terminology and registration systems used at the facility. The same requirements for competence should apply to persons conducting the procedures.

### Investigator

The investigator is responsible for the selection of the model, the experimental design including the definition of humane endpoints, the acquisition of raw data and materials, and scientific reporting. A basic system for the reporting of clinical signs will cater for unscheduled observations to be reported in a systematic way, which will allow for a serendipitous approach to research outcomes and timely collection of data and samples when a humane endpoint is reached. Furthermore, standardized reporting of clinical signs will allow for good statistical evaluation and further interpretation of this information. An investigator may draft a model-specific score list for the systematic recording of clinical signs as a scientific outcome. Such a score list may contain standardized terminology from the glossary but may also include more specific or different observations to be made.

### Veterinarian

Veterinarians attending to research animals should be trained in the laboratory animal medicine that applies to the species and research models in their care. The veterinarian will greatly benefit from good clinical assessments by technical staff and investigators who report on animals requiring closer attention. Also, the veterinarian can expand the clinical examination of the animal by including more specific diagnostic procedures. Finally, the veterinarian is responsible for translating clinical observations to diagnoses and for taking any measures needed. The overall outcomes of clinical reporting can be evaluated for any epidemiological trends in animal health and well-being, and any specific conditions that are relevant to the rest of the population. The veterinarian plays a crucial role in the education and training of technical and scientific staff, both on observational/examination skills and on the proper use of the institutional glossary and recording system.

Institutional veterinarians with a role in regulatory compliance who supervise the management of animal health and well-being, including the proper application of humane endpoints, should benefit from the reporting of clinical signs in terms of welfare (severity) evaluation.

## Education and training

Present guidance documents on education and training (such as Weiss et al.<sup>19</sup>) do not address skills in finding, examining and defining clinical signs specifically, indicating room for progress in this field. Furthermore, many scientists in biomedical science lack this type of training as part of their academic education, except for persons with clinical training in veterinary medicine, medicine or dentistry. Ethologists may be particularly skilled in noting behavioural signs but may not necessarily be skilled in their interpretation as a clinical condition. Thus, recognition and recording of clinical signs should receive due attention in laboratory animal science education. In addition to the acquisition of necessary skills, supplementary training is required in both clinical examination and the proper use of terminology in reporting. Members of the animal care staff interact with the animals on a daily basis and are familiar with the normal clinical presentation of the animals. Commonly occurring conditions are typically part of their professional experience. However, to apply terminology uniformly requires structuring and training. Well established institutional approaches can serve as the basis for tuition and education; but some practical training will be needed as well, in the acquisition of observation skills and in the proper use of terminology and means of registration. Typically, all direct users should be trained in the recognition and proper reporting of the signs listed for the institution or a specific study.

## Means of registration

### *General sheets*

The registration of clinical signs can be done using the daily log for an animal room or a specific group of animals (e.g. animals on a specific study). Each entry must be dated, and registration of the time of day may help to provide an estimate of the time of first occurrence/detection of the sign. This may also help to evaluate, for instance, behavioural features relative to the biological rhythm and the daily routines of the animals (e.g. before or after meal feeding). Any preferences for format will depend on the amount of data entry anticipated. For example, young healthy animals in stock will not generate many signs which need to be registered. For unscheduled observations, the institutional glossary needs to be locally available for reference purposes, and a format for registration needs to be provided, including all relevant items, e.g. the identification of an animal or a cage. In addition, any actions taken (such as reporting a case to the veterinarian) should be registered as well. It is important that any clinical cases that are brought to the attention of the investigator or

the veterinarian are followed up. Any system used should inform any observer of significant observations made recently. This can be based on easy access to an automated database, printouts of the data on animals that have been scored recently, or the maintenance of the score sheets in the animal room. An example of a weekly general score sheet for one animal is presented in Figure 2a. This sheet can be started upon the first clinical remark on a particular animal. In addition, cage labels may be used to signal any animals with significant clinical conditions. New cases, when first observed, should be registered using approved terminology and reported as required by institutional organization and procedures. It is not unusual for laboratories or institutions to initiate a program for the systematic observation and recording of clinical signs by retrospectively analysing random comments from their own program. Typically, some of these entries will need some clarification. Next, they can be clustered to bring similar remarks together and bring them under a common denominator. These can then be renamed to what the staff agree upon, and a try-out phase can be started to see if the consistent application of this terminology is possible for all users, and determine how many cases cannot be described using the terms of the initial listing. The in-house glossary will still be completely flexible at this time, but training and communication will lead to improvements and finalization of this basic listing. This process is listed in Table 2.

### *Score sheets – tailored for project*

Clinical score sheets for specific models cater for the registration of scheduled observations in specific studies at the instruction of the investigator. Such records will typically list the observations that will contribute to the outcome of the study, with optional qualifiers (such as graded scores) or integrated scores that are derived from grading or combining of scores. The score sheets may indicate a humane endpoint and should leave room (open slots) for unscheduled observations. Such sheets can be designed to record data on one animal, for one observation, or observation days, or for a longer period of time. As an example of a model-specific form, a weekly score sheet for a mouse after the induction of experimental autoimmune encephalomyelitis (EAE) is shown in Figure 2b. Alternatively, forms can be designed to cover all animals on a specific study for single or multiple observations. When automated systems are used, data entry can be direct or the written data can be transferred to an automated system later on. Written reports should be managed in a traditional way, e.g. it needs to be clear who is to retain the record for further use of reference and how long it should be archived. This issue will be less complex in



(a)

Clinical sign	Qualifier	Score	year = 20....						
			Date						
Weight loss	5%	1							
	10%	5							
	20%	20							
Vocalisation	none	0							
	Provoked vocalisation	5							
	Unprovoked vocalisation	10							
Chromo-dacryorrhea	None	0							
	Slight porphyrin staining	1							
	Excessive porphyrin staining	10							
Palpebral aperture	Open	0							
	Half closed	4							
	Sunken and half closed	10							
Hunching	Normal posture	0							
	Hunched posture	1							
Hypokinesia	Normal activity	0							
	Reduced activity	5							
	Inactive	5							
Piloerection	None	0							
	Piloerection	1							
Unscheduled sign	.....								
Unscheduled sign	.....								
Unscheduled sign	.....								
Total score	Add up points for the day								
Action taken	Who was consulted, what has been done								

**Figure 2(a).** General score sheet.

Example of a weekly clinical signs score sheet to record a set of non-specific clinical signs observed in small rodents where a severe condition may arise. Only non-specific signs are listed but unscheduled signs, specific or non-specific, may be added as they may occur. The personnel working with or responsible for the animals should be familiar with the listed signs and how they are defined and recognized. Unscheduled signs to be listed should be circumscribed, which is where the glossary may be helpful in defining the terminology and explaining the clinical condition. There may be a preference for listing a term in a common language, to be specifically linked to the technical term in the glossary. A score can be assigned to each qualifier and linked to a specific action, such as a humane endpoint (either a single score or after summing up to a total score, and also taking the duration and any exacerbation or reduction into account). The data from the clinical score sheet, and any scores adhered to, will contribute to the scientific outcomes and the application of animal welfare measures. How the terms link to the glossary: *chromodacryorrhoea* and *piloerection* listed as the technical terms; (half) closed eyelids = *blepharospasm*, sunken eyes may be associated with *dehydration* (see glossary for other signs); hunched posture = *kyphosis*; reduced activity = *hypokinesia*; inactive = *apathy* or *lethargy*.

**Figure 2(b).** Score sheet for experimental autoimmune encephalomyelitis (EAE) in mice (model-specific).

Example of a weekly clinical signs score sheet to record a set of clinical signs typically associated with EAE in mice. EAE is induced by active immunization targeting myelin proteins and is mostly studied as an animal model of human demyelinating diseases of the central nervous system, including multiple sclerosis (MS) and also acute disseminated encephalomyelitis (ADEM). Furthermore, it is used as a general model for T-cell mediated autoimmune disease. Demyelination leads to neurological impairment to be scored clinically. In addition, some non-specific signs are typically associated with EAE and scored as well (weight loss, reduced interaction with the environment). Separate and total scores contribute to the scientific data, are used to follow the development of EAE (increase and decrease) and are instrumental in setting criteria for a humane endpoint. How the terms link to the glossary: slow or rapid breathing are *bradypnoea* or *tachypnoea* respectively; passive behaviour = *apathy*; clumsy gait = *dysbasia*; the dragging of hindlimbs or reduced forelimb adduction are signs of *paresis*; *paralysis* is listed as the technical term.

(b)

Clinical sign	Qualifier	Score	year = 20....						
			Date						
<b>Appearance:</b>									
Body weight loss (compared to initial weight)	Up to 10% 10 to 20% 20 to 35%	1 2 3							
Coat condition	Slightly unkempt Lack of grooming Marked/prolonged piloerection	1 2 3							
<b>Body function:</b>									
Bladder incontinence	Evidence of some loss of control (small amount of urine in nest) More pronounced "leaking" of urine Incontinence	1 2 3							
Bladder retention	Palpated but will empty on handling More effort required to empty bladder Unable to urinate without assistance; signs of discomfort/distress during or after manual emptying	1 2 3							
Tail tone	Diminished lifting or curling of tail Loss of tone in distal half of tail Loss of tone in entire tail	1 2 3							
Respiration (rapid, slow or deep breathing)	Slight alteration Moderate alteration Marked alteration	1 2 3							
<b>Interaction with environment:</b>									
Nest condition	Slightly disorganised Some attempt at nest building but disorganised No nest	1 2 3							
Social behaviour	Reduced interaction with other animals Significantly reduced interaction, passive	2 3							
<b>Position and movement:</b>									
Side resting position	Present	3							
Righting time	Slow to right when placed on back Marked difficulty in righting Inability to right within 5 seconds	1 2 3							
Paresis	Slow forelimb abduction when placed on back Reduced forelimb abduction No forelimb abduction	1 2 3							
Gait	Clumsy Dragging one hind limb Dragging two hind limbs	1 2 3							
Paralysis	Present	3							
<b>Other observations:</b> (scores to be established)	..... .....								
<b>Total daily score:</b>									
<b>Actions taken:</b>	Who was consulted, what has been done								

Figure 2(b)

**Table 2.** Quick start.

---

Creating your own glossaries by a step-wise approach

1. Identify the areas of application
    - (a) Species
    - (b) Animals in colony (stock and/or breeding)
    - (c) Specific research models
  2. Find out what notes have been made in the past
  3. Cluster these notes to combine similar observations
  4. Discuss the analysis as a group, and agree
    - (a) What these descriptions actually describe
    - (b) Which clusters you select to translate to your initial glossary
    - (c) What items from the reference glossary would suit your purpose
    - (d) What common terminology will be used in connection with the scientific term
    - (e) Which qualifiers should be reported for each term
  5. Agree on one or more formats to start data collection
    - (a) Paper forms, per day or per (group of) animal(s)
    - (b) Simple generic software
  6. Train all users on
    - (a) The proper application of the glossary (use reference pictures if you have them)
    - (b) The use of the registration format
    - (c) Clinical observation and examination
  7. Celebrate the start of using the initial glossary
  8. Review and analyse the outcomes after a few months
  9. Discuss the outcomes and findings as a group
  10. Adapt your glossary/registration format accordingly
  11. Start thinking of making the system more capacious and/or efficient (e.g. the use of sophisticated automation).
- 

cases where automated records are made. Access by all users will be enhanced by keeping and updating documents in a shared folder, which will also facilitate the retention of records.

Project-specific lists will need to be first developed based on the clinical signs anticipated. In cases where painful conditions are likely to occur, typical species and model-specific clinical signs of pain should be included for systematic objective assessment and reporting.<sup>20–25</sup>

The form will evolve over time as some preprinted signs may never occur and others will need to be added based on their frequency.

## Automated databases

### Systems

Many automated systems for the management of colonies of laboratory animals or animal research have been developed in recent decades. Typical databases for animal management will use one record per animal containing fields for different types of data. In some systems, only animals in the study are registered; while in others, colony management, with or without breeding

records and pedigrees, needs to be catered for. Not all such systems have modules for the registration of clinical signs, veterinary care and other items relevant to the management of animal health and welfare and data acquisition. It is beyond the scope of this report to elaborate on the types of automated animal management systems. In general, two fields of application have been developed and are in use in many institutions. Firstly, the organized acquisition of data on clinical signs in toxicology studies has been carried out for decades and has been automated from the 1980s onwards. Secondly, breeding records based on individual animals have always been relevant to systematic inbreeding and outbreeding, but the application of breeding records has become far more widespread after the technology for the genetic modification of animals was developed in the same period. This technology has also prompted the more systematic phenotyping of such models of which clinical signs are a significant part, in conjunction with physiological assessments, pathology, etc.

Thus, it can be safely assumed that increasing numbers of animals are being recorded in automated systems based on unique identification. Modules for the registration of clinical signs have been used routinely in toxicology, but less so in (academic) research institutions or breeders' establishments. Further development in the near future is anticipated, and therefore the time is ripe to discuss the preferred features of such systems. This discussion should not deal with obvious needs for continuity such as backups of data and database configurations but should focus on the types of information to be managed for the recording and reporting of clinical signs.

### Animals

The unique record for each animal should contain specific information such as the species, strain, line, site and (approximate) date of birth, sex, etc. Any official or recommended nomenclature should be applied in the records, although this is not essential to the registration on the animal as such but to relate any data acquired to such information. The record number or animal number needs to be uniquely related to the animal identification attached to the animal or a defined small group of animals (such as animals in a litter), but these means of identification do not need to be the same. For instance a record number can point to a unique microchip number with a format that is entirely different. Also, records can be created and numbers can be assigned as animals are taken into a facility or into a specific study. It is necessary to consider which fields must be completed and which ones remain optional, and how rigid the system should be. For example in transgenics, several background strains are used frequently. However, crosses and backcrosses are made and it would be too rigid to

allow for only one background strain or just an F1 hybrid. It may be sensible to include the option 'mixed genetic background' with or without more specific reference to the strains that are in the animal's pedigree.

### *Record keeping*

It is important to consider how these records, and data entries to be added, will be managed. Which persons in the organization should have access to which records, with what type of authorization (read only, writing), and how will any changes be traceable? Should the system retain information on date and time when changes or additions have been made, and by which user? What should happen in cases where information is changed, e.g. should the older information be overwritten or should it be retained? How long and where should records be kept on animals that do not live anymore and studies become completed (please note, there are also legal requirements for this)? And who will need access to consult archived information, and will later additions or changes be allowed? In cases where the data management system is changed later, should these old data be readable using the new system or will the old management system be maintained for this purpose? Should the rights of a person who has left the organization be passed on to someone else, e.g. an investigator in the same research group? These are questions that need to be addressed upfront!

### *Writing and reading*

Information on clinical signs should be entered by the persons who check animal health and well-being on a daily basis, typically involving animal care staff, but should not be restricted to them. Persons conducting procedures, animal welfare officers and veterinarians should be allowed to add information as well. Furthermore, mistakes can occur so there needs to be a limited time window to make corrections. Typical examples would be confusion about the identity of the animal reported or the need to correct the terminology used.

Automated systems offer the flexibility to be used by different users and to link to other locations or documents. To bridge the different needs of different users, glossaries can be predefined, e.g. by pre-programmed lists of conditions observed frequently. This terminology can be bilingual which can enable animal care personnel to use words in their native language, and which can then be read and used in English by the investigator.

### *Notifications*

The system may or may not be used to notify specific persons of animals in need of attention. This option is

intrinsic to the use of a centralized automated system rather than a local or paper-based system. Information of people involved, or even active notification, can be organized by shared access to the same database, by notifications by email (manually or automated), or be left to the discretion of persons who have actual access to the animals or who are responsible for daily inspections. It is important to restrict this type of notification to new cases and cases that deteriorate clinically, otherwise the information will soon fail to draw attention. Also, notifications may be restricted to clinical signs that may reflect affected health or well-being, or even be related to the severity expected in a specific protocol. An estimate of severity is to be based on procedures and observations on health and well-being. Clinical signs need to be assessed in an objective way, and the use of terminology such as 'severe' should be avoided in reporting. The duration of a condition will be reflected in the number of days that it lasts. However, in case a humane endpoint has been defined for a specific study or model, the nature and duration of typical clinical signs can help to define this endpoint more precisely and to ensure it is applied accordingly.

### *Recent history*

Daily observations must be based on direct observations of the animals but also be related to observations made previously. As many observations will be made repeatedly over a longer time span (e.g. some sparsely haired areas), these should be copied easily for repeated reporting of the same observations. Defaults may be set to repeat those registrations until revoked. This will greatly simplify the registration process, thereby making it more time efficient and effective. Other types of observation are more likely to occur incidentally and may require repeated reporting.

### *Context*

Apart from observations of clinical signs on one animal, other remarks may be given on individual animals or groups of animals because they are relevant to health or well-being. This should be set up in a most careful way. Examples would be the incidental deviations from set climatic conditions in a room, or an event in a particular cage (e.g. a drained water bottle). Some remarks might concern an animal with a behavioural condition that affects other animals clinically, e.g. aggression, or barbering (if one mouse in a cage still has its whiskers it is suspect to be the barber). Events to be reported should deal with deviations from the norm, e.g. food left would be the normal situation for animals fed *ad libitum*, in which case the absence of food is worth reporting. Contrarily, however, food left may indicate lack of appetite of an animal that is fed

**Table 3.** Glossary of clinical signs.

Clinical sign	Definition	Clinical description	Detailed reporting	Background information
General signs				
<b>Oedema</b> <i>oedematous</i>	The abnormal accumulation of fluid in tissues.	Soft swelling of tissues, can be seen when it occurs in superficial tissues. Typically, oedematous tissue will 'pit' when gently compressed and the tissue will remain compressed for a while after withdrawal of external pressure.	Indicate the site(s) and size of the lesion and its chronicity.	Oedema can occur in a variety of organs and tissues secondary to a traumatic or pathological cause. Superficial oedema typically occurs locally after trauma or by a systemic cause in places that vary by species. Systemic causes include circulatory failure or low blood protein concentration (hypoproteinaemia).
<b>Distended abdomen</b>	An abdomen abnormally enlarged.	The enlarged abdomen extends beyond normal body contours (best seen from the ventral or dorsal side of the animal).	It must be determined if it is symmetrical or unsymmetrical, painful, rigid and (in larger animals) if there is a fluid thrill in percussion or not.	A distended abdomen is physiological in obesity or pregnancy. Pathological causes include fluid in the peritoneal cavity, intra-abdominal cysts or masses, enlarged organs, or bloated gastrointestinal tract.
<b>Ascites</b> <i>ascitic</i>	Abnormal accumulation of fluid in the peritoneal cavity characterized by a distended abdomen and fluid thrill upon percussion.	A distended abdomen and fluid thrill upon gentle percussion of the abdomen.	Must be differentiated from other causes of distended abdomen (pregnancy, solid mass). If differentiation is not possible, report 'distended abdomen'.	Ascites may result from a condition primarily affecting the peritoneum or may be secondary to liver, cardiac or kidney disease.
<b>Pallor (generalized)</b> <i>pale</i>	Pallor is an abnormally light colour of skin or mucosae.	Generalized pallor is typically symmetric and may affect all parts of the body. Paleness can be observed in mucous membranes, the unpigmented eyes of albino animals and in hairless areas.	Record the location where paleness was most clearly observed. Indicate the degree of pallor and the chronicity of the condition, as well as any secondary signs such as weakness, exercise intolerance, cold extremities and tachycardia.	Pallor demonstrates a lack of peripheral blood circulation or anaemia. Anaemia can be diagnosed by blood analysis which can also reveal the nature of the condition (excessive loss of red blood cells or lack of blood cell formation)

The glossary provides a structure for a glossary and an extended reference list for the creation of tailored glossaries. Page 6 of 31 is shown here for illustration purposes. The extended glossary will be maintained as a live document at the FELASA webpage ([www.felasa.eu](http://www.felasa.eu)).



**Table 4.** Clinical signs (alphabetical order).

Terminology from glossary in alphabetical order

Abortion	Dwarfism/Dwarf	Hypopigmentation	Pica
Agalactia	Dysbasia	Hypopyon	Piloerection
Aggression	Dyskinesia	Hypothermia	Plantigrade, walking
Agoraphobia	Dysmetria	Hypotony	Pollakiuria
Alopecia	Dysphagia	Hypotrichosis	Polydipsia
Anaesthesia	Dysphoria		Polyphagia
Analgesia	Dyspigmentation	Icterus	Polyuria
Anisocoria	Dyspnoea		Poor coat condition
Ankyloblepharon	Dysstasia	Kyphosis	Preputial discharge
Anorexia	Dystocia		Pruritus
Anuria	Dysuria	Lagophthalmos	Ptyalism
Apathy		Lameness	Pustule
Aphacia	Ecchymosis	Lethargy	
ArthropHYMA	Ectropion	Leukocoria	Rearing
Ascites	Emaciation	Leukoderma	Rectal prolapse
Ataxia	Enophthalmos	Leukotrichia	Regurgitation
Atocia	Entropion		Rhinorrhoea
Atony	Epileptic seizures	Macule	
	Epiphora	Malocclusion	Scab
Blepharoptosis	Epistaxis	Malodour	Scar
Blepharospasm	Erosion	Mass	Sneeze
Blindness	Erythema	Mastitis	Spasm, clonic
Bradycardia	Erythruia	Melena	Spasm, tonic
Bradypnoea	Exophthalmos	Microphthalmos	Strabismus
Buphthalmos		Miosis	Stranguria
	Food or water intake abnormal	Mydriasis	Subcutaneous emphysema
Cachexia			Synechiae
Cannibalism	Haematemesis	Nasal discharge	
Chromaturia	Haematochezia	Nystagmus	Tachycardia
Chromodacryorrhea	Haemoptysis		Tachypnoea
Circling	Haemorrhage	Obesity	Tenesmus
Coloboma	Hair dyschromia	Ocular hyperaemia	Tetraparesis
Coma	Halitosis	Oedema	Tetraplegia
Congenital malformation	Head intentional tremor	Oligodipsia	Trauma
Constipation	Head rotation	Oligophagia	Tremor
Coprophagia	Hemiparesis	Oliguria	Trismus
Corneal cellular infiltration	Hemiplegia	Onychopathy	Tumour
Corneal oedema	Hyperaemia	Ophthalmorrhage	
Corneal pigmentation	Hyperaesthesia	Opisthotonos	Ulcer
Corneal scarring	Hyperalgesia	Otitis (externa)	Unkempt hair
Corneal ulcer	Hyperexcitability		
Corneal vascularization	Hyperkeratosis	Pachyderma	Vaginal discharge
Cough	Hyperkinesia	Pallor (generalized)	Vaginal prolapse
Crust	Hypermetria	Papule	Vesicle
Cyanosis	Hyperpigmentation	Paralysis	Vocalization
	Hyperthermia	Paraparesis	Vomit or emesis

(continued)

**Table 4.** Continued

Terminology from glossary in alphabetical order			
Deafness	Hypertony	Paraplegia	
Dehydration	Hyphaema	Paresis	Wound
Diarrhoea	Hypoaesthesia	Petechia	
Distended abdomen	Hypoalgesia	Photophobia	Xeroderma
Distichiasis	Hypokinesia	Phthisis bulbi	Xerophthalmia

restrictedly (with meals). Thus, what and how to report should be worked out carefully to be relevant to the situation at hand, and personnel should be trained to make observations and report accordingly with sufficient knowledge of the biology and the normal husbandry of the species in mind.

## The glossary

### *Terms, definitions, descriptions and qualifiers*

Many examples of glossaries exist, and for different purposes, but they tend to be tailored to specific types or animals or studies. Also, many glossaries are company-owned and have not been published, although some good examples are available in the public domain (such as OECD mono 19<sup>16</sup> or [www.mousewelfareterms.org](http://www.mousewelfareterms.org)<sup>26</sup>). The Working Group has used both public and corporate documents and has chosen to draft long lists of possible observations, organized by organ (systems) (Table 3). These lists are intended to be of help, and are not meant to be prescriptive, although reference to a published glossary will facilitate definition and harmonization.

This glossary was drafted anticipating the capture of unscheduled observations, and the terminology may be too shallow for the study of specific organ systems, yet be too detailed for the management of a healthy breeding colony. It is subdivided into sections: general, behavioural, neurological, gastrointestinal, respiratory/circulatory, urogenital, ophthalmic, and integumentary signs. This subdivision is arbitrary, and some of the terms could fall into more than one category, but these divisions categorize the list and make it more user-friendly. An alphabetical list for cross-referencing is added in Table 4. In most cases, the scientific veterinary/medical terminology is used and these technical terms can easily be found in (veterinary) medical dictionaries, which can also be used to find definitions phrased in languages used locally. When choosing a term to use, this can be replaced by a more readily translatable one, thereby bridging language differences (e.g. cyanosis could translate into 'bluish' in any language). The columns to the right provide a definition

and a clinical description, respectively. For reporting the condition, there is an option of allowing more detailed reporting by adding relevant qualifying terms, such as the location on the body. Finally, some background information can be given, e.g. a reference to normal physiology in some species, possible associations of signs with other or systemic diseases, common causes, or what further diagnostics may be applied.

The glossary is meant to be applicable to all mammalian species commonly used for research. The terminology can be adapted for use in birds (replacing 'hair' by 'feathers' and 'parturition' by 'laying', among other species-specific features). However, the glossary is not a veterinary dictionary nor does it offer information on the anatomy and physiology of the different species. We gladly refer to a wealth of information available on the biology of the various species (also handbooks which are easily accessible to hobbyists), and on the husbandry of the species in a research context. More specific information on the pathology, differential diagnosis, diagnostic confirmation and options for the management or treatment of conditions are found in general veterinary textbooks or specific references for laboratory animals.

Both a typical example from the glossary (Table 3) and the alphabetical listing (Table 4) are included in this report proper. The glossary will be managed as a live document on FELASA's website (<http://www.felasa.eu>) by the authors of this report as an editorial committee.

### *Translation of clinical signs to severity*

There is no validated desktop approach to relating a randomly captured clinical sign, or a combination of signs, to the classification of severity of a condition. Clearly, some conditions are perceived to be more alarming than others, or justify immediate veterinary attention. The need to consult with a veterinarian upon first observation may be indicated on a score sheet or may result in automatic notification when a sophisticated database system is in place. However, to classify severity one needs to interpret the combined observations to make an overall assessment, and also the duration of the condition should be taken into account.

This situation is different for specific research models. For some the course of events can be predicted better and the pathology may be well documented. Thus, (sets of) clinical signs can be used to define scientific and humane endpoints based on onset, development and duration of the relevant sign(s), and a fair assessment of severity can be made. It is important to keep in mind that the clinical condition of the animal is one aspect of severity, and procedures are another. Typically, many procedures will not cause significant signs upon observations made the next day. The severity of a procedure should be assessed based on both experimental procedures and outcomes, and the experience of an animal should be evaluated over a longer period of time.

### Acknowledgements

The Working Group wishes to acknowledge FELASA for venturing into this field and to thank the companies and academic institutions that have offered unpublished glossaries and score sheets for our consultation and use.

### References

1. LASA (Laboratory Animal Science Association)/APC (The Animals Procedures Committee). *Report of working group to examine the feasibility of reporting data on the severity of scientific procedures on animals*. Tamworth, Staffordshire: LASA, 2008. [http://www.apc.gov.uk/reference/lasa\\_apc\\_final\\_report.pdf](http://www.apc.gov.uk/reference/lasa_apc_final_report.pdf).
2. 2010/63/EU Directive of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, Official Journal of the European Union L 276/33. [http://ec.europa.eu/environment/chemicals/lab\\_animals/legislation\\_en.htm](http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm) and Commission Implementing Decisions 2012/707/EU and 2014/11/EU.
3. Fentener van Vlissingen JM, Kuijpers MHM, Van Oostrum ECM, Beems RB and Van Dijk EJ. Retrospective evaluation of clinical signs, pathology and related discomfort in chronic studies. In: Hendriksen CFM and Morton DB (eds) *Humane endpoints in animal experiments for biomedical research*. London: Laboratory Animals Ltd, 1999, pp.89–94.
4. Carstens E and Moberg GP. Recognizing pain and distress in laboratory animals. *ILAR J* 2000; 41: 62–71.
5. Stokes WS. Reducing unrelieved pain and distress in laboratory animals using humane endpoints. *ILAR J* 2000; 41: 59–61.
6. Canadian Council on Animal Care (CCAC). *Guidelines on choosing an appropriate endpoint in experiments using animals for research, teaching and testing*. Ottawa, Ontario: CCAC, 1998. [http://www.ccac.ca/en/CCAC\\_Programs/Guidelines\\_Policies/GDLINES/ENDPTS/g\\_endpoints.pdf](http://www.ccac.ca/en/CCAC_Programs/Guidelines_Policies/GDLINES/ENDPTS/g_endpoints.pdf) (accessed 10 January 2015).
7. Toth LA. Defining the moribund condition as an experimental endpoint for animal research. *ILAR J* 2000; 41: 72–79.
8. Morton DB. A systematic approach for establishing humane endpoints. *ILAR J* 2000; 41: 80–86.
9. Wallace J. Humane endpoints and cancer research. *ILAR J* 2000; 41: 87–93.
10. Dennis MB. Humane endpoints for genetically engineered animal models. *ILAR J* 2000; 41: 94–98.
11. Olfert ED and Godson DL. Humane endpoints for infectious disease animal models. *ILAR J* 2000; 41: 99–104.
12. Henriksen CFM and Steen B. Refinement of vaccine potency testing with the use of humane endpoints. *ILAR J* 2000; 41: 105–113.
13. Sass N. Humane endpoints and acute toxicity testing. *ILAR J* 2000; 41: 114–123.
14. Brown MJ and Murray KA. Phenotyping of genetically engineered mice: humane, ethical, environmental, and husbandry issues. *ILAR J* 2002; 47: 118–123.
15. National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. Working document on genetically altered animals, [http://ec.europa.eu/environment/chemicals/lab\\_animals/pdf/corrigendum.pdf](http://ec.europa.eu/environment/chemicals/lab_animals/pdf/corrigendum.pdf) (2013, accessed 10 January 2015).
16. OECD (Organisation for Economic Co-operation and Development). Guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation. ENV/JM/MONO 19. Paris: OECD, 2000. <http://www.oecd.org/ehs/test/mono19.pdf>.
17. Fox JG, Anderson LC, Loew FM and Quimby FW (eds) *Laboratory animal medicine*, 2nd ed. Amsterdam: Elsevier Inc, 2002.
18. Pritchett-Corning KR, Girod A, Avellaneda G, Fritz PE, Chou S and Brown MJ. *Handbook of clinical signs in rodents and rabbits*. Wilmington, MA: Charles River Laboratories, 2010.
19. Weiss J, Bukelskiene V, Chambrier P, et al. FELASA recommendations for the education and training of laboratory animal technicians: Category A. *Lab Anim* 2010; 44: 163–169.
20. Hawkins P. Recognizing and assessing pain, suffering and distress in laboratory animals: a survey of current practice in the UK with recommendations. *Lab Anim* 2002; 36: 378–395.
21. National Research Council. *Recognition and alleviation of pain in laboratory animals*. Report of the NRC Committee on Recognition and Alleviation of Pain in Laboratory Animals. Washington DC: National Academies Press, 2009. <http://www.nap.edu/catalog/12526.html>.
22. Langford DJ, Bailey AL, Chanda ML, et al. Coding of facial expressions of pain in the laboratory mouse. *Nat Methods* 2010; 7: 447–449.
23. Wright-Williams S, Flecknell PA and Roughan JV. Comparative effects of vasectomy surgery and buprenorphine treatment on faecal corticosterone concentrations and behaviour assessed by manual and automated analysis methods in C57 and C3H mice. *PLoS One* 2013; 8: e75948.

24. Gigliuto C, De Gregori M, Malafoglia V, et al. Pain assessment in animal models: do we need further studies? *J Pain Res* 2014; 7: 227–236.
25. Faller KME, McAndrew DJ, Schneider JE and Lygate CA. Refinement of analgesia following thoracotomy and experimental myocardial infarction using the Mouse Grimace Scale. *Exp Physiol* 2015; 100: 164–172.
26. Bussell J, et al. Mouse welfare terms, <http://www.mousewelfareterms.org> (last modified 16 September 2014, accessed 10 January 2015).