European Commission

Caring for animals
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DIRECTIVE 2010/63/EU
ON PROTECTION OF ANIMALS USED FOR SCIENTIFIC PURPOSES

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SEVERITY ASSESSMENT FRAMEWORK

Environment

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National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes

Working document on a severity assessment framework

Brussels, 11-12 July 2012

The Commission established an Expert Working Group (EWG) for the assessment of severity of procedures to facilitate the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes. All Members States and main stakeholder organisations were invited to nominate experts to participate in the work.

The EWG for the assessment of severity met twice: in December 2011 with the focus on genetically altered animals, and in May 2012 discussing a general framework for assessing the actual severity experienced by animals in procedures.

This document is the result of the work of the two EWG meetings, discussions with the Member States as well as legal input from the Commission on the understanding of a severity assessment framework, its components, participants and working tools and methods. It was endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU at their meeting of 11-12 July 2012.

The table of contents:

The related articles of Directive 2010/63/EU

- **Article 4(3)** "*Member States shall ensure refinement of breeding, accommodation and care, and of methods used in procedures, eliminating or reducing to the minimum any possible pain, suffering, distress or lasting harm to the animals*."
- **Article 15(1)** "*Member States shall ensure that all procedures are classified as 'non-recovery', 'mild', 'moderate', or 'severe' on a case- by-case basis using the assignment criteria set out in Annex VIII.*"
- **Article 16(1)(d)** " *it [reuse] is in accordance with veterinary advice, taking into account the lifetime experience of the animal."*
- **Article 54(2)** "*Member States shall collect and make publicly available, on an annual basis, statistical information on the use of animals in procedures, including information on the actual severity of the procedures and on the origin and species of non-human primates used in procedures. …*"

General background

Directive 2010/63/EU on the protection of animals used for scientific purposes requires that a prospective assessment is made on the severity of each procedure in a Project (Article 15) and that a severity classification is assigned, which may be either "non-recovery", "mild", "moderate" or "severe". Annex VIII provides guidance on the factors to be taken into account in the consideration of prospective severity and provides some examples in each severity category.

Article 54 on reporting requires that for statistical information, the actual severity of the pain, suffering, distress or lasting harm experienced by the animal must be reported (in contrast to the prospective assessment, or prediction, of severity made at the time of the project evaluation). In addition, the actual severity of any previous procedures will be a key consideration in determining whether or not an animal can be reused in further procedures (Article 16).

These measures provide opportunities to improve the quality of science and welfare through prospective review of project proposals and, by inclusion of the actual suffering experienced by the animal, should provide greater transparency and understanding of the impact of scientific procedures on animal welfare.

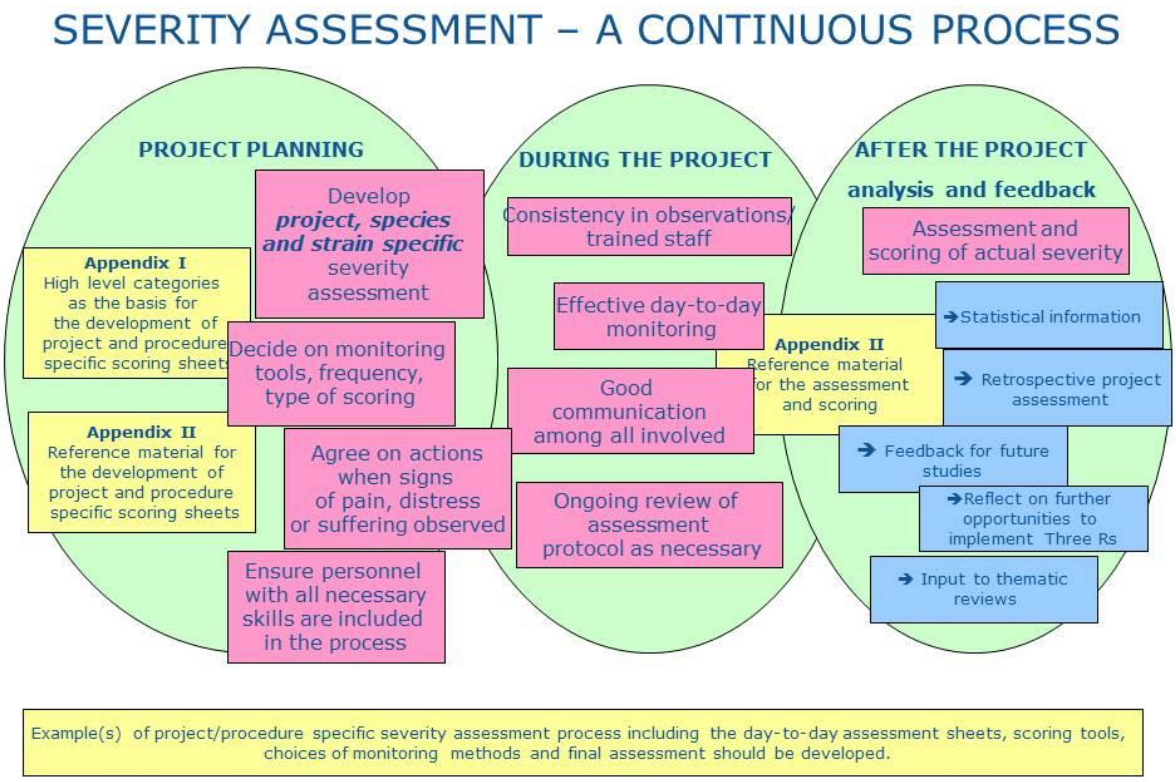
Main benefits of prospective assessment, monitoring, assessing and recording actual severity include

- Opportunities in particular to implement Refinement and reduce suffering, although prospective discussions will generally also provide an opportunity to consider whether or not animal use is necessary (Replacement) and the study design is appropriate to minimise animal use (Reduction);
- Improved animal welfare, e.g. if suffering is recognised and alleviated sooner;
- Improved transparency, as statistics should better reflect the actual welfare costs to animals;
- Improved communication between those responsible for using, caring for and monitoring animals;
- Input to retrospective project assessment when this is carried out (Article 39);

- Improved scientific data quality due to better welfare;
- Increased knowledge about assessing severity and clinical signs, which will promote greater consistency in assessments – provided that approaches and results are disseminated, e.g. via journals, discussion groups and meetings;
- Input into training courses for researchers, animal technologists and laboratory animal veterinarians, if results are used to provide examples;
- Evidence-based information that can be used in prospective harm-benefit assessments for similar, future projects.

General considerations for a severity assessment

The consideration of severity within a procedure should be a **continuous process** beginning with initial study design, through the study-specific day-to-day monitoring of animals during the project, to the "actual" severity assessment upon completion of the study, which provides opportunities to identify further refinements for future studies.



By approaching in this manner, there is a greater opportunity to ensure that the Three Rs are considered and implemented throughout, that communication among all involved will be improved and that consistency will be enhanced.

Effective severity assessment requires

- **A 'team' approach,** with input from people with different expertise, experience and priorities, e.g. researchers, animal technologists and care staff, the attending veterinarian;
- Good planning;
- Appropriate continuing education and training of all personnel involved;
- Day-to-day severity assessment systems that are appropriately tailored to the species, strain and project, including informed and structured observations of animals at appropriate intervals (e.g. frequency increased during and after procedures);
- Well-informed, effective protocols for assessing behaviour and clinical signs;
- Analysis of the observations to make an informed judgement on the nature and level of suffering;
- Awareness of the severity of each procedure and what action to take if this is reached or exceeded;
- A consistent approach to overall judgements on actual suffering (mild, moderate, severe) for statistical reporting;
- Reflection upon how effectively the Three Rs were implemented and whether improvements could be made for future studies.

Pre-study considerations

The process for ensuring that severity is minimised during scientific procedures begins at the **design stage,** when considering whether or not it is necessary and justified to use live animals to meet the scientific objectives.

- Where the use of live animals is necessary and justified, it is important to ensure that an appropriate model is chosen and that the study design is robust;
- All aspects of the study that may cause pain, suffering, distress or lasting harm should be identified, and consideration given to how their effects can be minimised, for example by consulting the literature, colleagues, animal technologists, the veterinarian and the Animal Welfare Body if appropriate;
- The recommended prospective severity classification assigned to procedures should be based on the highest severity anticipated for any animal on the study;
- A plan for observing the animals that is suitable for and tailored to the study should be developed. A standardised terminology that can be understood by all those involved in the study will improve consistency in reporting and interpretation;
- It is important to ensure there are sufficient trained and competent staff available to conduct the study, and monitor and care for the animals.

Indicators of severity

There are behaviours and clinical signs that may be used to assess the severity of procedures at the 'cage side' (or tank, pen etc.). The terminology used to describe these should be understandable by all those involved in the use, monitoring and care of the animals. For any severity assessment system, a sound understanding of the normal health, behaviour and welfare status of the species (strain, where applicable) being observed is essential.

The aim should be to:

- achieve the best possible quality of life for the animal;
- ensure that any suffering due to the scientific procedures is recognised and
- minimised, but

to remain consistent with the scientific objectives.

Any assessment system should effectively detect deviation from a normal state of health and welfare, enabling the observer to record and convey a clear, consistent assessment of each animal.

A simple, hierarchical approach can be used to define a severity assessment protocol that is appropriately tailored to the species, strain, individuals and procedure. The process for defining a cage side assessment protocol should identify any adverse effects that may occur throughout the animal's lifetime experience, including housing, husbandry, care and handling, as well as adverse effects due to the scientific procedures and their consequences. Consideration of all these adverse effects should identify indicators that can be used to effectively assess the animal's wellbeing at the cage side. These indicators should be tailored to the species, strain and experimental procedures being applied. They should also be easy to understand, to identify and to record consistently. However, it is important to ensure that there is also the facility to capture and record any unexpected adverse effects, for example in free text.

High level categories

A set of overarching, 'high level' categories that apply across all species is listed below as a starting point for producing a comprehensive list of specific indicators for each procedure or animal care programme. The aim is to produce a study-specific list of sufficient indicators to minimise the risk of missing signs of suffering, without devising an overly complex system that will be unnecessarily bureaucratic and time-consuming.

The high level categories are:

- Appearance
- Body functions
- Environment
- Behaviours
- Procedure-specific indicators
- Free observations (other relevant observations)

Indicators within each of these categories can be adapted to any species. They should be used to produce a list of observable characteristics that can be assessed by a suitably trained individual, in order to make a judgement on the overall health and welfare status of the animal.

These indicators should be discussed and selected in liaison with the person(s) responsible for oversight of the welfare of the animals, and the Animal Welfare Body if appropriate. They should then be used to develop study-specific cage side record keeping systems for day-to-day observation, monitoring and assessment.

Appendix I provides an example of how these high level categories can be further sub-divided and used to develop suitable observational criteria, using common descriptive terminology.

Appendix II provides information on guidelines and online resources that can assist in the development of appropriate welfare assessments for animals undergoing scientific procedures.

Factors that should be considered in the assessment of actual severity

It is important to note that depending on the specific situation, a number of elements may have a positive or negative impact on severity, and species differences need to be taken into consideration.

The assessment of actual severity should be undertaken on an individual, case-by-case basis, using the observations taken from the animals during day-to-day monitoring. Additional parameters required for study purposes can also be used where appropriate and available. For example, non-observable indicators (such as body temperature, body weight, biochemical parameters or biotelemetry data such as heart rate) may also be needed for the study and should be taken into account in the assessment of severity if they can provide additional, relevant information.

The actual severity to be reported for the individual animal should be the highest level experienced during the course of the procedure and not based on the severity at the end of the procedure. Nor should the evaluation be considered a simple additive process e.g. a number of mild procedures = moderate severity. It should be based on an overall assessment of the animal's experience from the start of the procedure to the end.

The list below provides examples of the kind of <u>elements to be considered and weighed</u> when assessing actual severity.

<u>Procedure, technique</u>

- Surgical / non-surgical;
- Level and duration of restraint;
- Withholding analgesia/anaesthesia when either or both of these would otherwise be necessary;
- New model or procedure;
- Environmental elements (including housing and food/water restrictions);

- Stress /distress;
- Repeated procedures and intervals between these (also need to consider frequency and combination of "below threshold" interventions);
- Reuse or continued use.

Species, strain, stage of development, previous experience

- **This should be a major consideration – it is necessary to understand the biology and behaviour of the species and strain (and sometimes individual) to be able to predict and assess severity effectively;**
- **Species and strain;**
- Origin of the animal, e.g. purpose-bred, feral or wild;
- Sourcing (including previous housing conditions) and transport;
- Genotype, phenotype, sex, age, immune status;
- Natural behaviour and biology (e.g. the relative importance of different senses, such as sight for primates and smell for rodents, and how these may be affected in a laboratory environment);
- Single/group housing - justification to singly house social animals, or to separate them from established groups in the short or long term;
- Diurnal rhythms, e.g. impact of conducting scientific or husbandry procedures on nocturnal animals during the light phase;
- Maternal separation in all species, including rodents;
- Cognitive ability, awareness, memory, perception of effects of procedures.

Frequency, intensity

- There is no direct link between frequency and severity, i.e. increased frequency does not necessarily result in greater severity. This is because the effect on severity of repeating procedures or techniques depends on a number of factors, such as the intensity of each intervention, its duration, the species and the experience of the individual;
- When interventions are repeated, there is the potential for acclimatisation, which may reduce severity, e.g. in a non-human primate undergoing mild procedures. Conversely, repetition may increase severity, e.g. due to anticipation of a stressful procedure, or onset of hyperalgesia if surgery is involved;
- Potential for positive reinforcement training, or 'rewards' following procedures;
- The highest level of severity should be recorded instead of 'recovery level' severity.

Duration of effect

- Duration is *linked* with intensity (*and therefore with severity*);
- Whether it is possible to use early humane or scientific end-points.

Effectiveness of refinements

- Appropriate analgesia, anaesthesia and post-operative care;
- Enrichment – both environmental enrichment and group housing of social animals;
- Housing, husbandry and care – whether it is possible to refine these according to current best practice, or whether the procedure necessitates restrictions such as confinement to smaller enclosures (e.g. metabolism cages), grid flooring or exposure to environmental conditions that could cause stress;
- Training the animal to cooperate, or facilitating habituation to procedures;
- Effectiveness of cage side assessment protocols.

Cumulative severity

- Each animal's whole-life experience, in which restrictions on the ability to refine housing, or need for frequent capture, handing and restraint etc. may affect severity, must be taken into account within a procedure that involves a number of steps/ interventions;
- Previous procedures, in the case of reuse.
- The life-time experience, including elements such as sourcing (e.g. early 'weaning') and transport, is required to be taken into account when reuse is being considered.

How to ensure consistency in the assessment and assignment of actual severity

Input at the study design phase by relevant scientists, animal technologists, veterinarians and care staff is generally needed to ensure that there are appropriate data available to enable an informed decision on actual severity at the end of the procedure. The final assignment of an actual severity category will be the result of an analysis of records of cage-side observations of behaviour, clinical signs and other relevant parameters.

Elements contributing to consistency include:

- Incorporation of multiple expertise, experience and priorities – a 'team approach';
- Training in using the day-to-day assessment protocol (including the common terminology used to describe observations);
- Expertise on animal health, welfare and behaviour;
- Regular review of outcomes;
- Communication between all those responsible for conducting the study and monitoring the animals (top-down, bottom-up, between and within);
- Oversight (locally (e.g. the Animal Welfare Body), regionally, nationally, EU).

The following key issues should be considered to ensure consistency in the assessment of actual severity:

Development of a procedure specific assessment sheet

- Assessment sheets that are tailored to the species, strain and study should be developed and agreed prior to the start of the project;
- All available, relevant information should be used effectively in the development of study-specific assessment sheets, for example previous experience, results of *in vitro* or *in silico* studies, literature searches, information from pilot studies and observed clinical signs in humans or other animals;
- Information on which parameters need to be observed and how the monitoring should be carried out should be available at the cage side;
- The prospective severity level classification will partly 'dictate' the level of involvement needed at the operational level, whether a team approach is required during the monitoring, and who should be involved in the actual observations and recording process. Those who developed the study-specific assessment protocol should carry out and/or confirm the actual, final severity assignment;
- Depending on the complexity of the study, separate assessment sheets for separate components may be helpful e.g. standard surgery/peri-operative care sheet used in combination with tailored study protocol assessment;
- In some cases, the study-specific assessment sheets may also need to include information relevant to colony management e.g. GA animal breeding and growth data.

Consistency in actual severity assessment

Assessment of actual severity is conducted at the end of the procedure and requires a judgement to be made on the overall severity actually experienced by the animal, on the basis of the day to day assessments and taking into account the procedures that were conducted.

- One commonly used approach is to define ‘mild’, ‘moderate’ and ‘severe’ levels for each of the indicators used in the day to day assessments, and then make a judgement about the severity of these on a case by case basis;
- **As with the day-to-day monitoring, it is essential that the actual assessment criteria are tailored to the procedure, species and strain;** e.g. a 10 % loss in body weight will have very different implications for the health and welfare of a juvenile, growing rat; an adult mouse with a rapidly growing tumour; or an adult dog.
- Consideration of the time period over which some of these indicators occur is also an essential factor, particularly with respect to weight loss and food/water consumption.

Assessment will be made by using the daily assessment records, taking into account the procedure performed on the animal, how long adverse effects lasted and whether or not the animal was reused. Although this will inevitably involve a certain degree of subjectivity, proper training of the observer should aim to reduce such subjectivity.

Assigning actual severity if animals are found dead

- If an animal is found dead, i.e. not euthanised, this may be either as a consequence of the experimental procedure, or other unrelated causes[1];
- The actual severity for animals found dead should be reported as 'severe' unless an informed decision can be made that the animal did not experience severe suffering prior to death;
- If it is unlikely that death was preceded by severe suffering, the actual severity classification should reflect the known experience prior to death. Factors such as frequency of monitoring, use of analgesia, etc. will need to be given due consideration.;
- "(lasting) harm" can only be experienced by a living animal.

Examples to illustrate the process of severity classification, day-to-day assessment and final, actual severity assessment should be developed and made available to the scientific community.

Who should provide input for the actual severity assessment?

- Observation and recording of effects are often separate processes from the actual severity assignment;
- Clear responsibilities should be set to ensure effective day to day monitoring of the animals, with the appropriate support and oversight;
- A verification process should be in place to promote consistency, e.g. by comparing assessment scores made by different people;
- Roles with respect to observing and monitoring animals and making the actual severity assessments should be flexible and adjustable on the basis of the complexity and severity of the study in question – although the legal responsibility for ensuring that suffering is detected and minimised remains with the person named in the project authorisation;
- Animal Welfare Bodies should also play a role at establishment level to ensure consistency;
- The National Committees and Competent Authorities may also contribute to promoting consistency.

[1] For the purposes of statistical reporting, actual severity should primarily relate to the severity of the experimental procedures and not unrelated incidents such as disease outbreaks or cage flooding. These types of incident relate to health problems or to husbandry and care practices, not harms due to procedures, however, they should recorded, investigated further and followed up to prevent recurrence.

Monitoring tools, media and other considerations

- The use of score sheets should be considered at the project planning stage;
- Score sheets should be as simple as possible, but as detailed as needed, and tailored to the type of study;
- Previously developed assessment sheets can be used if these are appropriate to the study, species and strain;
- Electronic record keeping can help to ensure consistency and ease of access for all relevant information;
- The use of standardised language and terminology is recommended;
- The data recorded should be as objective as possible;
- The advantages and disadvantages of (i) numerical scoring and (ii) 'binary' (where indicators are marked as 'present' or 'absent') observation systems should be considered on a case-by-case basis;
- All types of observation record should include a facility to add free text, as well as predetermined indicators, so that unexpected observations can be recorded;
- Effective training for all relevant staff is essential covering specifically severity and welfare assessment as well as monitoring techniques;
- A communication plan should be established to encompass all relevant staff; this should include a mechanism to rapidly communicate unexpected outcomes to all appropriate individuals and, as applicable, to the Competent Authority;
- Monitoring should be proportionate to anticipated effects – procedures that may cause 'severe' suffering will generally require more frequent and detailed monitoring;
- There should be clear criteria for intervention, for example, if particular parameters are observed or if a predetermined level of suffering is approached. All relevant staff should know what these criteria are, know what to do and whom to contact should they occur.

If the severity assessment process is implemented effectively, the animals and all personnel involved in their care and use will benefit from improved animal welfare, scientific validity and transparency.

Good internal and external communication on the severity assessment process and on the application of the Three Rs will afford even wider benefits.

Appendix I

Glossary of Clinical Observations

The success of any severity assessment scheme depends upon the selection of welfare indicators that:

• are readily and reliably recognisable;

• are effective at providing good measures of welfare;

• are relevant to the scientific study, species and strain (where appropriate);

• are practical to carry out and do not overly disturb the animal and

• lend themselves to consistent measurement, interpretation and analysis.

A common approach to recording clinical observations is therefore a desirable goal as this will help in the development of consistent approaches to severity classification. This would facilitate comparisons of clinical findings between studies, and inform those involved in severity assessment.

The observations are structured on the following six high level categories:

Appearance / Body Functions / Environment / Behaviours / Procedure-specific indicators / Free observations

High level categories	Areas to focus on when observing animals	Specific indicators to monitor
Appearance	**Body condition**	Weight loss/gain
		Obese
		Thin
		Body condition score, if available
	Coat and skin condition	Piloerection
		Unkempt/lack of grooming

		Greasy coat
		Hair loss
		Dehydration – skin tenting
		Skin lesions – swelling; scab; ulcer; injury/wound
		Faecal or urine staining
	Discharge	Ocular; nasal; uro-genital; porphyrin staining in some species e.g. rat
	Eyes	Sunken or 'dull'
		Closed/semi-closed
		Damage/injury to eye (e.g. corneal ulceration)
	Mouth	Salivation
		Malocclusion/overgrown teeth
	Other	'Pain face' – e.g. semi-closed eyes and nose bulge in mice
		Abdominal constrictions
		Swollen body part, e.g. distended abdomen
Body functions	**Respiration**	Accelerated breathing (tachypnoea)
		Laboured breathing (hyperpnoea)
		Very laboured breathing (dyspnoea)
		Wheezing or other sound when breathing
	Food/water intake	Increased/decreased
	Body temperature	Increased/decreased; measured body temperature if available (e.g. via microchip or telemetry device, contact or non-contact thermometry); colour of extremities in rodents
	Senses	Impaired sight, hearing or balance
Environment	**Enclosure environment, including any litter, nesting material, enrichment items**	Presence and consistency of faeces
		Wet bedding, e.g. due to polyuria
		Presence of vomit or blood
		Whether animal is using enrichment items e.g. nesting material, chew blocks
Behaviours	**Social interaction**	Change from normal temperament - apprehensive/aggressive interactions with other animals; anxiety (e.g. marked escape responses, hiding)
		Isolated or withdrawn from other animals in social group

	Undesirable behaviours	Repetitive/ stereotypic behaviour
		Barbering (rodents), trichotillomania
		Increased aggression to humans or other animals
	Posture and mobility	Abnormal posture
		Abnormal gait; lameness; lack of movement/lethargy/reluctance to move if stimulated
		Uncoordinated movements
		Hunched abdomen; tilted head
	Other	Tremors
		Seizures/convulsions/spasms
		Vocalisation; spontaneous or invoked. *(Note - Some species, e.g. rodents, usually vocalise in the ultrasonic range, so audible vocalisations are of special concern. Rabbit vocalisations are also generally inaudible to humans unless the animal is in distress).*
Procedure-specific indicators	These are identified on the basis of the individual project, its potential adverse effects and expected indicators of these	For example, in an EAE model these could include; loss of tail tone, hind limb weakness, fore limb weakness, paralysis, loss of bladder function
Free observations	A severity assessment scheme should always include a facility to note any observations of unexpected negative welfare impacts.	

Appendix II

Background reading, guidelines and online resources on assessing the welfare of animals undergoing scientific procedures

American College of Laboratory Animal Medicine (ACLAM) (2006) *Guidelines for the Assessment and Management of Pain in Rodents and Rabbits*, download at http://www.tinyurl.com/65ez5vh

Assessing the Health and Welfare of Laboratory Animals (AHWLA) training resource. See http://www.ahwla.org.uk/index.html

Canadian Council on Animal Care (CCAC) *Welfare assessment*. See http://www.ccac.ca/ and click on the Three Rs microsite, then search for 'welfare assessment' (English or French)

Categorising the severity of scientific procedures on animals - Summary and reports from three round-table discussions edited by Jane A. Smith and Maggy Jennings on behalf of the Boyd Group and the RSPCA, July 2004
Published by RSPCA Research Animals Department

FELASA Working Group on the Reporting of Clinical Signs in Laboratory Animals (2012) – (in press)

Institute for Laboratory Animal Research (ILAR) (2008) *Recognition and Alleviation of Distress in Laboratory Animals*. Washington, DC: National Academies Press
Institute for Laboratory Animal Research (ILAR) (2009) *Recognition and Alleviation of Pain in Laboratory Animals*. Washington, DC: National Academies Press. See http://dels.nas.edu/animal_pain/

Johansen R, Needham JR, Colquhoun DJ, et al. (2006) Guidelines for health and welfare monitoring of fish used in research. *Laboratory Animals* **40:** 323–340

Joint Working Group on Refinement (2011) A guide to defining and implementing protocols for the welfare assessment of laboratory animals. *Laboratory Animals* **45:** 1-13

Leach MC et al. (2008) Identification of appropriate measures for the assessment of laboratory mouse welfare. *Animal Welfare* **17:** 161-170

National Centre for the Three Rs (NC3Rs) *Welfare assessment*. See http://www.nc3rs.org.uk/welfareassessment

National Health and Medical Research Council (2008) *Guidelines to Promote the Wellbeing of Animals Used for Scientific Purposes: The Assessment and Alleviation of Pain and Distress in Research Animals.* Canberra: Australian Government. See http://www.nhmrc.gov.au and search for "pain and distress"

Organisation for Economic Co-operation and Development (OECD) (2000). *Guidance Document on the Recognition, Assessment, and Use of Clinical signs as humane endpoints for experimental animals used in safety evaluation.* OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 19. Paris: OECD

Wells DJ, Playle LC, Enser WEJ, et al. Assessing the welfare of genetically altered mice. Full version at http://www.nc3rs.org.uk/gamice. Summary in *Laboratory Animals* **40:** 111–114

Workman P et al. (2010) Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer* **102:** 1555-1577, download at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2883160/?tool=pubmed

1.2 Suggested useful journals for further reading

Applied Animal Behaviour Science	http://www.applied-ethology.org/applied_animal_behaviour_science.html
Animal Technology and Welfare	http://www.iat.org.uk/publications/atw.htm
Animal Welfare	http://www.ufaw.org.uk/animal.php
Contemporary Topics in Laboratory Animal Science and *Journal of the American Association for Laboratory Animal Science*	http://www.aalas.org/publications/index.aspx#ct
Lab Animal and *Lab Animal Europe*	http://www.labanimal.com/laban/index.html http://www.labanimaleurope.eu/
Laboratory Animals	http://la.rsmjournals.com/

1.3 Suggested keywords for literature searches

The following keywords are helpful when searching for information on severity assessment:

affect	harm benefit assessment	positive indicators	severity scale
animal welfare	humane endpoints	positive welfare	sickness behavio(u)r
animal suffering	Needs	qualitative behavio(u)r assessment	stress
assessment	objective assessment	quality of life	suffering
discomfort	Pain	refinement	welfare assessment
distress	pain assessment	score sheets	welfare indicator
harm assessment	pain measurement	scoring system	welfare outcomes

References relating to actual severity classification

CCAC (1998) *Guidelines on: Choosing an Appropriate Endpoint in Experiments Using Animals for Research, Teaching and Testing.* Canadian Council on Animal Care, available at: http://www.ccac.ca/en_/standards/guidelines (English) and http://www.ccac.ca/fr_/normes/lignes_directrices (French)

FELASA Working Group on Pain and Distress (1994) Pain and distress in laboratory rodents and lagomorphs. *Laboratory Animals* **28:** 97-112

Jones HRP, Oates J and Trussell BA (1999) An applied approach to the assessment of severity. In: Hendriksen CFM. & Morton DB (eds), *Humane Endpoints in Animal Experiments for Biomedical Research*. Proceedings of the International Conference, 22-25 November 1998, Zeist, The Netherlands. Royal Society for Medicine Press Ltd., London pp 40-47

LASA /APC (2008) *Final Report of a LASA/APC Working Group to Examine the Feasibility of Reporting Data on the Severity of Scientific Procedures on Animals*. Available at: http://www.lasa.co.uk/publications.html

Morton, DB and Hau J (2011) Chapter 18: Welfare assessment and humane endpoints. In *Handbook of Laboratory Animal Science, 3rd Edition, Volume 1 Essential Principles and Practices.* Hau J and Schapiro, SJ (eds), CRC Press LLC, USA, pp 535-572

Prescott MJ, Morton DB, Anderson D, Buckwell A, Heath S, Hubrecht R, Jennings M, Robb D, Ruane B, Swallow J and Thompson P (2004) Refining dog husbandry and care, Eighth report of the BVAAWF/FRAME/RSPCA/UFAW Joint Working Group on Refinement. *Laboratory Animals* **38 Suppl 1:** S1:1-S1:94

Wolfensohn S and Lloyd M (2003) *Handbook of Laboratory Animal Management and Welfare, 3rd Edition*. Blackwell Publishing Ltd, Oxford (4th edition in prep)

(All URLs last viewed 24 May 2012.)

Examples to illustrate the process of severity classification, day-to-day assessment and actual severity assessment

Brussels, 11 January 2013

The *Working Document on a Severity Assessment Framework* produced by the European Commission Expert Working Group and endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU on the protection of animals used for scientific purposes at their meeting of July 2012 recommended that examples be developed to illustrate the "process of severity classification, day-to-day assessment and final, actual severity assessment" and that these should be made available to the scientific community.

Following on from this, the Expert Working Group produced a range of examples to show how the process described in the *Working Document* might be applied to different procedures. These are intended to help Competent Authorities, users, animal technologists, veterinarians and all other relevant staff to ensure that pain, suffering and distress are effectively predicted, recognised, ameliorated, where possible, and consistently assessed during procedures. This document was endorsed by the National Competent Authorities for the implementation of Directive 2010/63/EU at their meeting of 23-24 January 2013.

It is crucial that a number of important factors are taken into account when using these examples:

- It is assumed that **good practice is implemented** throughout with respect to housing, husbandry and care; refining procedures; education and training; assessing competence; retrieving and applying current information on replacement, reduction and refinement; and experimental design.

- The kind of score sheets included within the examples are intended to **complement – not substitute for – the judgement of trained, competent, empathetic staff.** The aim is to enable more systematic and objective observation, record keeping and assessment of suffering, but not to over-ride professional judgement.

- Each example relates to a **hypothetical, but realistic, situation.** It would not be appropriate to include all the detail that would be available in practice, but sufficient details are included **to explain how the process was applied.**

- As stated in the *Working Document*, it is essential to **think through and tailor severity assessment** to the species, strain and procedure as conducted at the individual user establishment. On that basis, the Expert Group **strongly advises against using** the tables and score sheet systems in the examples as they are, **even for the same procedures**. All severity assessment protocols should be regularly reviewed for effectiveness, and revised as necessary.

- The **examples are also subject to revision,** as knowledge increases about indicators of pain, suffering and distress and as approaches change to assessing and classifying severity. Each is labelled with a date; please check the EC website http://ec.europa.eu/environment/chemicals/lab_animals/interpretation_en.htm for updates.

- **Feedback would be welcome** on the usefulness of the examples, and suggestions for further procedures to be included; please send comments to env-laboratory-animals@ec.europa.eu

Table of contents:

Illustrative examples of the severity process
Model 1 – Oncology Studies (1a and 1b)

Last updated: 05 February 2013

1. Animal Models in Oncology Studies (1a and 1b)

General context: Evaluation of novel anti-cancer agents in vivo

Cancer is a major cause of death in the developed world and the aging of the human population will inevitably lead to an increase in the burden of disease. In 2010, the probability of cancer-related death in the EU before the age seventy was around one in seven. There is therefore a need to develop new, more effective drugs for the treatment of cancer. Benefits will involve reducing cancer mortality and improving the quality of life for those who develop cancer in the future.

Animal models are currently used in the development of new drugs for the treatment of cancer, in addition to computer modelling and in vitro methodologies such as cell culture assays. Once the selectivity and activity of compounds have been confirmed *in vitro*, only those compounds that exhibit favourable characteristics are tested in animals. Tolerability studies are performed with small groups of animals to establish the maximum tolerated dose (MTD) and suitability of dosing regimen prior to larger efficacy studies.

The severity of the effects on the animals will be dependent on the models and the purpose of the study. For example, the maintenance of tumour cell lines should not have a significant impact on welfare provided that good practice is observed throughout including appropriate animal monitoring and the adoption of early humane end-points. However, studies to assess novel treatment in metastatic models are likely to have more significant welfare concerns due to multiple tumour development and the likely adverse effects of cytotoxic drugs.

A number of guidelines for the welfare and use of animals in cancer research have been published, for example in the British Journal of Cancer (Workman et al. 2010). These provide a detailed overview of the various animal tumour models which are available, how these impact on the animals and how suffering can be minimised.

Two examples are provided here which illustrate oncology animal models of different severity classifications.

Reference

Workman et al. (2010) Guidelines for the welfare and use of animals in cancer research. *British Journal of Cancer* (2010) 102(11), 1555 – 1577; free download at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2883160/pdf/6605642a.pdf

1 (a) - Maintenance of Human Tumour cell lines in immunocompromised nude mice

Some human tumour cell lines do not replicate reliably in culture and there is the occasional need to characterise and maintain human cell lines in a xenogeneic *in vivo* model.

Study

30 male BALB/C nude mice will be subcutaneously injected on the left flank with 10^3 HCT 116 cell suspension in 0.1 ml saline. Animals will be group housed in Individually Ventilated Cages (IVCs) with litter and nesting material. Animal welfare will be assessed daily and animals weighed every 4 days. Individuals will be palpated for tumours every other day, and any detectable tumours will also be measured with callipers every other day. Animals will be euthanased on day 15 for tumour harvesting.

Initial prospective assessment and consideration of specific refinements and humane end-points

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might make it worse?**	**How will suffering be reduced to a minimum?**	
	Adverse effects	**Methodology and interventions**	**Endpoints**
Maintenance of immunocompromised mice	Animals are susceptible to infection	Housed in IVCs and husbandry practices tailored to minimise risk of contamination Animals group housed and environmental enrichment provided to reduce stress Husbandry and care will be reviewed if any signs of distress, aggression or abnormal behaviours observed	Any animal showing signs of ill-health will be killed
Sub-cutaneous injection of tumour cells	Transient discomfort following injection	Injection performed once only Appropriate volume will be injected (maximum of 0.2ml) Animals will be closely monitored during immediate post injection period	Animals will be humanely killed if more than mild distress or discomfort, without rapid recovery, observed following injection (very rare)

Growth of tumour	May cause discomfort or affect normal behaviour or locomotion Tumour may become infected or ulcerate (but should not metastasise)	Tumour growth will be measured every other day Monitoring scheme will include careful observation of posture, gait and tumour size and condition	Animal will be killed if tumour ulcerates, or interferes with normal behaviour, posture or locomotion, or exceeds 1.2cm in diameter (Workman et al. 2010)

Analysis

Animals are expected to experience only **MILD** discomfort and will be killed if any health or welfare problems arise above this level.
A prospective severity classification of MILD is therefore appropriate.
An example of a completed observation sheet is included at the end of this model

Clinical observations

A basic score sheet was drawn up that focused on tumour size, body weight, posture and gait, because few other clinical signs were expected. Space was included for unexpected clinical signs to be recorded as free text. An entry of NAD (no abnormality detected) confirms animals have been checked and no abnormalities noted. An example is included below.

Results

- No significant weight loss was recorded in any of the animals.
- In 5 animals no tumour development was noted.
- In 25 animals tumours developed on the flank. These tumours did not interfere with normal behaviour, and measured a maximum of 1 cm on Day 14 when animals were euthanased in accordance with the study protocol for tumour harvesting
- Some aggressive behaviour and fighting occurred in one cage; one animal had bite wounds on the tail and back and was separated in an individual cage, wounds were locally disinfected daily until healed and animal was kept until the end of the procedure.

Assessment of Actual Severity

- 29 animals completed the study with no more than mild suffering related to the injection and growth of tumours.
  Actual severity for these was considered to be **MILD** 1 animal had bite wounds which were effectively managed. In this animal, there was some additional suffering caused as a consequence of aggression, but this was unrelated to the procedure. These incidents were dealt with effectively and suffering minimized. Although the level of suffering experienced by this animal was moderate, as this incident was unrelated to the procedure, the actual procedure-related severity to be reported was considered to be **MILD**

Example observation sheet (completed for hypothetical case)

Tumour Growth in Nude Mice – Procedure & Observation Sheet				
Cage 1 – Mouse numbers 1-5				
Date	Procedure	Tumour size (cm)	Weight (g)	Clinical Observations - **check posture and gait carefully**
28/02	s.c. injection		1- 21 2- 22 3- 21 4 -22 5- 22	No signs of welfare problems following injections
01/03				No Abnormality Detected (NAD)
02/03	Palpation			NAD
03/03				NAD
04/03	Palpation		1- 21 2- 22 3- 21 4 -22 5- 22	NAD
05/03				NAD
06/03	Palpation			NAD
07/03				Some aggressive behaviour; no wounds apparent
08/03	Tumour measuremen	1 – 0.1 2 – 0.1	1- 21 2- 22	Mice 1 had bite wounds on tail and back – local treatment; moved to single housing. Nest box provided

	t	3 – 0.1 4 – no tumour 5 – 0.2	3- 21 4 -22 5- 22	for singly housed animal but removed from cage with remaining four mice in case this was triggering aggression
09/03				Wounds disinfected for mouse 1, healing well; no signs of aggression between remaining animals
10/03	Tumour measuremen t	1 – 0.2 2 – 0.1 3 – 0.1 4 – no tumour 5 – 0.2		Wounds disinfected for mouse 1
11/03				Wounds disinfected for mouse 1
12/03	Tumour measuremen t	1- 0.4 2 – 0.3 3 – 0.3 4 – no tumour 5 – 0.5	1- 22 2- 22 3 - 21 4 -21 5- 23	Wounds healed for mouse 1, disinfection discontinued.
13/03				NAD
14/03	Euthanase and harvest tumour.			

1 (b) Efficacy of novel pharmaceutical agents on tumour growth - Multi-step procedure

The study is intended to assess the efficacy of novel agents at reducing or arresting growth of tumour cells. The tumour needs to be well established before treatment can begin (usually 0.5 cm in diameter is sufficient) – due to the duration of the study some tumours may develop up to a maximum of 1.2 cm in diameter, usually in the vehicle control group. Cytotoxic drugs are likely to cause some adverse welfare effects.
30 male BALB/C nude mice will be injected with slowly growing tumour cells (0.1 ml). Animal welfare will be assessed daily and animals will be weighed once a week for 3 consecutive weeks. Tumour growth will measured with callipers on day 7 and day 14; on day 20, tumours will be measured again, animals will be randomized and treatment started in the form of twice daily intra-peritoneal injections for 7 days.

Initial prospective assessment and consideration of specific refinements and humane end-points

What does this study involve doing to the animals?	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects	**Methodology and interventions**	**End-Points**
Maintenance of immunocompromised mice	Animals are susceptible to infection	Housed in IVCs and husbandry practices tailored to minimise risk of contamination Animals group housed and environmental enrichment provided to reduce stress Husbandry and care will be reviewed if any signs of distress, aggression or abnormal behaviours observed	Any animal showing signs of inter-current disease will be killed
Sub-cutaneous injection of tumour cells	Transient discomfort following injection	Injection performed once only Appropriate volume will be injected (maximum of 0.2ml) Animals will be closely monitored during immediate post injection period	Animals will be humanely killed if more than mild distress or discomfort, without rapid recovery, observed following injection (very rare)
Growth of tumour	May cause discomfort or affect normal behaviour or locomotion Tumour used may become infected or ulcerate (but should not metastasise)	Daily observation of animals, regular monitoring of general health and tumour growth Monitoring scheme will include careful observation of posture, gait and tumour size and condition Pharmaceutical interventions will begin when tumour reaches 0.5 cm in diameter (measured by callipers)	Animal will be killed if tumour ulcerates, or interferes with normal behaviour, posture or locomotion, or exceeds 1.2cm in diameter (Workman et al. 2010)
Intraperitoneal injection	Transient discomfort following injection	Animals will be closely monitored	Animals will be killed if weight loss

of novel pharmaceutical agent	Cytotoxic drugs may cause diarrhoea, weight loss, anorexia or lethargy	during immediate post injection period Maximum volume of 10ml/kg daily for 7 days Minimum dose levels will be used (determined following dose ranging studies) Clinical scoring system will be used to assess welfare	exceeds 20% of initial body weight Animals not eating or having diarrhoea for more than 48 hours will be killed An upper limit for a clinical score will be set as a humane endpoint

Analysis

As a consequence of the tumour size, the increased potential for ulceration, the frequency of injections and the adverse effects of the drugs given, a **prospective severity classification of MODERATE** is appropriate in this case.

Could the severity limit be MILD?

Most unlikely, unless the scientific objectives could be attained with earlier end-points, for example reducing the maximum tumour size. It would also imply injection of drugs at a dose known not to cause any significant adverse clinical effects. Under these circumstances a **MILD** severity could be considered appropriate.

Clinical Observations
An example of an observation sheet and a sample score sheet are included at the end of this model

Results

Of the 30 male BALB/C mice, 25 were used for efficacy evaluation; 10 animals received drug B at dose H, 10 drug B at dose X and 5 drug C at dose Y;

Assessment of actual severity

- 3 animals did not develop tumours and were euthanized as unusable for the experiment - **MILD**
- 2 animals developed ulceration at the tumour injection site before treatment started and were euthanased. – **MODERATE**

- 10 animals receiving drug B at dose H had tumours that remained relatively small, with no significant BW loss and no clinical signs – **MILD**
- 7 animals receiving drug B at dose X had a decrease in tumour size, a BW loss of 15% and presence of loose stools, but were kept until the end of the experiment - **MODERATE**
- 3 animals receiving drug B at dose X had a decrease in tumour size, a BW loss of 15%, presence of loose stools, anorexia and were very lethargic; these were humanely killed on day 25 – **SEVERE**
- 5 Animals receiving drug C at dose Y had a continued increase in tumour size, body weight increased, no clinical signs apart from tumour growth. These animals were euthanised when the tumour size exceeded 1.2 cm - **MODERATE**

Example of a score sheet

Animal no.				
Date	01/06	02/06	03/06	04/06
Appearance				
Body weight				
Coat condition				
Body function				
Dyspnoea and/or tachypnoea				
Food intake				
Environment				
Loose stools or diarrhoea				
Blood in diarrhoea				
Behaviours				
Handling				
Aggression				
Abnormal gait				
Abnormal posture				
Reluctance to move				
Procedure-specific indicators				
Tumour size				
Ulceration of tumour				

Tumour impeding movement				
Total score				
Any other observations				

Examples of clinical scores

Appearance	**Score**
Bodyweight	
5-10% weight loss	1
11-15 % weight loss	2
16-20% weight loss	3
20% + weight loss	HEP
Coat Condition	
Coat slightly unkempt	1
Slight piloerection	2
Marked piloerection	3
Body Function	
Tachypnoea (fast breathing)	1
Dyspnoea (difficulty breathing)	3
Environment	
Loose stools or diarrhoea	1
Blood in diarrhoea	HEP
Behaviour	
Tense and nervous on handling	1
Markedly distressed on handling, e.g. shaking, vocalizing, aggressive	3
Locomotion	
Slightly abnormal gait/posture	1
Markedly abnormal gait/posture	2

Actions	
Score 1	Review frequency of monitoring
2	Consider supplementary care, *e.g.* extra fluids
4	Consult veterinarian
6	Implement humane endpoint

Significant mobility problems / reluctance to move	3
Immobility >24h	HEP
Procedure Specific Indicators	
Tumour size >1.2cm	HEP
Tumour ulceration	HEP
Tumour impeding movement	HEP

Illustrative examples of the severity process
Model 2 – Experimental Autoimmune Encepyhalitis (EAE) in mice

Last updated: 05 February 2013

General context

Experimental Autoimmune Encephalomyelitis (EAE) is used to model various aspects of Multiple Sclerosis (MS) in rodents and primates. MS is a multiform, complex neurological disorder that occurs in young adults. Its symptoms include inflammation, demyelination and axonal loss. Animal models are used to research the physiopathology of this disease, and to evaluate potential protective or curative strategies, including immunomodulation, immunoprotection, axonal regeneration and myelin repair. The multiform-multiphasic characteristics of MS require that appropriate models be used to address specific questions relating to different stages of the disease.

EAE involves generating immune system activity targeted at myelin, which induces inflammation in the central nervous system and opening of the blood brain barrier. This can cause a severe neurological syndrome in the animal model, which should be followed by a partial recovery during the first chronic remitting relapsing phase. This phase is associated with inflammation and reversible demyelination. After 9-10 weeks, the animal will enter the progressive form, which is associated with chronic demyelination and axonal loss. During this phase it is possible to evaluate different therapeutic strategies. Humane and scientific endpoints must be carefully chosen, taking the aims of the study into account.

References

Emerson MR *et al.* (2009) Enhancing the ability of Experimental Autoimmune Encephalomyelitis to serve as a more rigorous model of Multiple Sclerosis through refinement of the experimental design. *Comparative Medicine* **59:** 112-128
Miller SD *et al.* (2010) Experimental Autoimmune Encephalomyelitis in the mouse. *Current Protocols in Immunology.* **88:** 15.1.1 – 15.1.20
Weissert R (ed) (2012) *Experimental Autoimmune Encephalomyelitis - Models, Disease Biology and Experimental Therapy.* Published by In Tech, DOI: 10.5772/1190, http://www.intechopen.com
Wolfensohn S *et al.* (in prep) Reducing suffering in Experimental Autoimmune Encephalomyelitis.

Study

In this example, EAE will be induced in four male and four female Biozzi ABH mice (a widely used strain that is believed to have a high translational value), in order to evaluate a potential therapy for MS. At the initial project planning stage, the user considers each possible adverse event for the animals and identifies potential causes of suffering, in discussion with the animal technologist and care staff and attending veterinarian. They researched refinements and these are implemented in the project. The mice will be socially housed in single-sex groups of four. Particular attention will be paid to the local

environment, as animals with EAE will have significant motor deficits. Cages will be provided with solid flooring, sawdust litter, adequate refuges and nesting material, and chew blocks. Animals will be treated with an inflammatory adjuvant to induce EAE and monitored during recovery and the chronic remitting relapsing phase (9 to 10 weeks). Once the progressive form has developed, candidate therapeutic compounds will be evaluated in the mice in a three week study.

Initial prospective assessment and consideration of specific refinements and humane end-points

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might make it worse?**	How will suffering be reduced to a minimum?	
	Adverse effects	**Methodology and interventions**	Endpoints
Multiple injections of inflammatory adjuvant	Discomfort or pain due to priming injection Possible reaction at injection site, causing irritation or discomfort	Small doses will be injected into multiple subcutaneous sites (not footpads or tail base) The adjuvant and vehicle are formulated so as to be minimally irritant Animals will be monitored following injection	Animals will be humanely killed if more than transient moderate pain or distress observed after injection
Induction of EAE – initial severe neurological syndrome followed by recovery phase	Paralysis, which may cause distress or anxiety: loss of tail tone, hind limb weakness, hypo-motility, limb paralysis Urinary dysfunction (incontinence or retention)	Urinary function will be monitored by checking the bladder daily. The bladder will be expressed manually when necessary in cases of retention (monitoring carefully for signs of pain or distress following bladder emptying) If animals are incontinent, cage will be frequently checked for damp litter and nesting material; replaced with fresh materials as necessary Adequate refuges and nesting material will be provided	HEP for any one of the following criteria: • Bilateral forelimb paralysis for >24h • Bilateral hindlimb paralysis for up to 5 days • Any self mutilation • Persistent urinary retention/Inability to empty bladder • Paresis (loss of movement; slight paralysis)

	Significant weight loss (e.g. up to 35 %)	Constant access will be ensured to water and food placed in containers on the cage floor Body weight and condition will be monitored daily and scored more frequently (as necessary) once weight loss had begun Soaked food and fluid blocks will be provided, with subcutaneous supplementation when necessary	• Weight loss of 35 %* • Ceasing to eat or drink for >24h after the onset of the disease • Non-recovery from EAE 3 weeks after onset of clinical disease • Clinical signs of intercurrent disease e.g. hunching
Remitting/relapsing clinical course	Chronic neurological deficits	All stressors will be reduced, including noise levels Ambient temperature will be raised as necessary, using heating pads, extra litter and nesting material	
Administration of novel therapeutic agent (during progressive form)	Discomfort due to injection Side effects or lack of efficacy of agent	Animals will be monitored closely following injection of candidate agent	Animals will be humanely killed if any of the above indicators are observed, or if there are any severe side effects due to the novel therapeutic agent

* Weight loss of 35 % is an extreme endpoint that requires sound scientific justification. In this case, significant weight loss is unavoidable and the animals can recover from this with appropriate support, e.g. supplementary warmth and additional feeding, including hand feeding if necessary. The endpoint of 35 % is set for this particular study as a way of reducing the requirement to induce EAE in further naïve animals, which would be significantly higher with a more 'conventional' endpoint (e.g. 20 %).

Analysis

A **prospective severity of SEVERE is deemed appropriate** as the procedure is expected to cause severe impairment of the animals' general wellbeing and condition.

Could the severity be MODERATE?

Although prospective severity of this model should always be SEVERE for the reasons outlined above, the retrospective severity classification may be MODERATE depending on duration of study and implementation of early HEP as indicated here.

Clinical observations

During the study mice were monitored by the animal technologists and care staff using a clinical score sheet system that had been tailored to the protocol following discussion with the users, animal technologists and care staff and veterinarian). This included parameters relating to weight, fur condition, tail tone, bladder control, righting, gait, paresis and advanced signs (side resting position; near complete paralysis; rapid, slow or deep breathing). As the project involved severe procedures, animals were very closely monitored and ongoing reviews of severity were regularly conducted by the user, in discussion with the Animal-Welfare Body, animal technologist and designated veterinarian. An illustrative example of a score sheet is shown below.

Example of an appropriate score sheet

Table. Clinical score sheet used for EAE mice

Date:					
Appearance					
Body weight					
Coat condition					
Body function					
Bladder control					
Tail tone					
Respiration					
Environment					
Nest condition					
Behaviour					
Social behaviour					
Gait					
Procedure-specific indicators					
Side resting position					

Righting time					
Paresis					
Paralysis					
Other observations					
(Free text)					

Notes: Each indicator was assessed according to the system in the table below, in which (for example) '1' would be entered into the table next to 'tail tone' if diminished lifting were observed, and '2' next to 'nest condition' if the nest were disorganised.

Table. Assessment system for indicators in EAE clinical score sheet

Score:	**1 = Mild**	**2 = Moderate**	**3 = Severe**
Weight loss	Up to 10 %	10 to 20 %	20 to 35 %
Coat condition	Slightly unkempt	Lack of grooming	Marked /prolonged piloerection
Bladder control - incontinence	Evidence of some loss of control, e.g. small amount of urination in nest	More pronounced 'leaking' of urine	Incontinence
Bladder control - retention	Bladder can be palpated but will empty on handling	Slightly more effort required to empty bladder	Unable to urinate without assistance; signs of discomfort/distress during or after manual emptying
Tail tone	Diminished lifting or curling of tail	Loss of tone in distal half of tail	Loss of tone in entire tail
Respiration: rapid, slow or deep breathing	Slight	Moderate	Marked
Nest condition	Slightly disorganised	Some attempt at nest but disorganised	No nest
Social behaviour	No change expected with mild suffering; scoring begins at 2	Reduced interaction with other animals	Significantly reduced interaction; passive
Gait	Clumsy	Dragging one hindlimb	Dragging two hindlimbs
Side resting position	No change expected with mild or moderate suffering; scoring begins	No change expected with mild or moderate suffering; scoring begins	Present

	at 3	at 3	
Righting time	Slow to right when placed on back	Marked difficulty in righting	Inability to right within 5 seconds after placing on back
Paresis	Slow forelimb abduction when placed on back	Reduced range of forelimb abduction when placed on back	No forelimb abduction
Near complete or complete paralysis	No change expected with mild or moderate suffering; scoring begins at 3	No change expected with mild or moderate suffering; scoring begins at 3	Present

Assessment of actual severity

At the end of the procedure, the score sheet was reviewed for each individual to see how highly the indicators had scored and how this had changed over time.

- Two mice lost 8 % of their body weight following induction of EAE , had slightly unkempt fur and slow forelimb abduction, but scored '2' for all other indicators for the first 5 days of the project. Their scores then reverted to '1' or '0' for each indicator for the relapsing/remitting phase and during the drug trial. Severity = **MODERATE**
- Three mice lost between 22 and 32 % of their body weight and scored a combination of '3', '2' and '1' throughout the relapsing/remitting phase and during the drug trial. Severity = **SEVERE**
- One mouse lost 37 % of his body weight during the post-induction phase and was humanely killed. Severity = **SEVERE**
- Two mice lost 15 and 18 % of their body weight respectively, and scored a combination of '2' and '3' for all other indicators for the first 4 days of the study. They then scored a combination of '1' and '2' throughout the relapsing/remitting phase and during the drug trial. Severity = **SEVERE**

Paralysis was not observed and it proved to be too difficult to assess breathing at the cageside level, so both of these were deleted from the record sheets. Increased time in the refuge was frequently noted in the free text boxes as an early indicator of suffering, so this was added to the sheets for future projects.

6 animals considered as **SEVERE**, 2 animals considered as **MODERATE**

Opportunities for further application of 3Rs

Following their assessment of actual severity, the users consulted with colleagues and searched the literature for further refinements. The following additional refinements were identified:

- Pre-feeding animals with high-energy supplement foods, such as jelly and condensed milk, before administering the adjuvant
- Using a lower dose of adjuvant
- Using an alternative study protocol so that the duration of the project could be reduced

These were added to the protocol for future studies, with the intention of comparing actual severity levels to see whether the refinements had been effective.

Illustrative examples of the severity process
Model 3 – Arthritis

Last updated: 05 February 2013

General context

Animal models of arthritis are used to study the pathogenesis of the disease and to evaluate potential anti-arthritic drugs for clinical use. Important criteria for model selection therefore include morphological similarities to human disease, and the capacity of the model to predict efficacy of candidate therapeutic compounds in humans.

Commonly used animal models of rheumatoid arthritis include: rat adjuvant arthritis, rat type II collagen arthritis, mouse type II collagen arthritis and antigen induced arthritis in several species (Bendele, 2001). Injection at the base of the tail is commonly used as it provides good immunogenic response, although other injection sites are also reported in the literature. There are also considerable strain variations with respect to susceptibility, severity and latency to onset of arthritis. For example, the susceptibility of Genetically Altered (GA) lines to the development of arthritis may be modified (enhanced or suppressed) dependent on the effects of the gene alterations. In animal models of arthritis that have been frequently used and are thus well validated, disease onset will be predictable and evaluation techniques are likely to be well defined and characterised. In such models multiple evaluations, including gait analysis and use of Von Frey filaments, may be used as opposed to single observational measures.

Note that regular reviews of the available strains, protocols and refinements should be undertaken so that the most appropriate one(s) are selected for the scientific question being asked on a case by case basis (Joe et al, 1999).

The model presented in this example is Type II Collagen arthritis in rats, which can cause severe suffering. Therefore, compelling scientific justification for its use is an absolute requirement. Rats are immunized against heterologous type II collagen, producing lesions that are similar to those seen in human rheumatoid arthritis (Bendele, 2001). The resulting polyarthritis is characterized by marked cartilage destruction associated with immune complex deposition on articular surfaces, bone resorption and periosteal proliferation, and moderate to marked synovitis and periarticular inflammation.

References

Bendele, A.M. Animal models of rheumatoid arthritis, J Musculoskel Neuron Interact 2001; 1(4):377-385

Jasemian Y et al. (2011) Refinement of the collagen induced arthritis model in rats by infrared thermography. *Br. J. Med. & Med. Res.* **1(4):** 469-477

Joe, B., Griffiths, M.M., Remmers, E.F., Wilder,R.L. Animal models of rheumatoid arthritis, Current Rheumatology Reports 1999 ; 1 139-149

Study

In this example, arthritis will be induced in 18 male and 18 female Lewis rats by repeated injection of FIA (Freund's Incomplete Adjuvant) and collagen. The injection site will be the base of the tail. Daily treatment will start 10 days later (D10), when arthritis will have developed, and will be then continued daily for a further 14 days (until D24). The aim of the study will be to test putative therapeutic agents. Previously published data on related compounds were reviewed to see whether providing analgesia would interfere with the scientific objectives, and it was established that this would introduce experimental confounds. Analgesia will therefore not be provided during the development of arthritis or to controls, and special attention will be given to non-pharmacological methods of pain relief (e.g. husbandry refinements) in order to ameliorate suffering.

All animals will be observed and weighed daily and scored on a general clinical score sheet, and will be tested on D0 (before the first injection) and on D10 (before start of treatment), D13, D16, D20 and D24. Testing will include indirect measures of impairment of physical function such as joint diameter (measured with callipers) and clinical scoring according to an arthritis scoring system. Humane endpoints will be applied on the basis of clinical scores (see below).

Evaluation of novel therapeutic pharmaceutical agents in a rat model of arthritis (type II collagen)
SEVERE Severity

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might make it worse?**	**How will suffering be reduced to a minimum**	
	Adverse effects	**Methodology and interventions**	**Endpoints**
Subcutaneous injections of bovine type II collagen in Freund's Incomplete Adjuvant (FIA) at the base of the tail on up to three occasions	Restraint stress Transient pain, moderate swelling at injection site and discomfort for one to two days Skin ulceration possible but very unlikely with FIA	Empathetic attitudes and competent handling throughout the procedures Standardised dose and formulation chosen to minimise swelling and pain	If skin ulceration persists or becomes infected, animals will be humanely killed
Development of arthritis (D0-D10)	Discomfort, pain, disability and distress; animal may show signs of ill health including dull appearance, inappetance, reluctance to move, weight loss, joint swelling, audible vocalisation on handling	Careful clinical monitoring using a general clinical scoresheet, with increased frequency of monitoring at onset of clinical signs (usually from around D8-D10) Additional soft litter and nesting material provided throughout the study Easy access to water and food (e.g. on floor of cage) throughout the study Arthritis clinical scoring system will be used, which assesses degree of swelling and the number of joints affected	Animals will be humanely killed when they reach the pre-determined clinical scores for humane endpoints (see table below)
Administration of pharmacological agents (test and control, twice daily) by subcutaneous or intraperitoneal route (from D10 to D24)	Transient discomfort following injection Pharmacological agents are not expected to cause adverse effects, based on previous animal data	Daily careful clinical monitoring using a general clinical score sheet	Humane endpoints will be applied if there are significant adverse effects
Evaluation of effects of	Depending on the methods used there	Careful clinical monitoring	See table below

pharmacological agents on severity of arthritis (D0, D10, D13, D16, D20 and D24)	may be some additional transient pain or discomfort e.g. use of Von Frey hairs, use of callipers, requirement for handling	Reduce frequency of monitoring (to the minimum consistent with scientific objectives) until animal recovers	

Note
A clinical and arthritis scoring system should be discussed by the investigator, veterinary surgeon and animal technologists and care staff, and agreed prior to commencement of the study.

Analysis

As a consequence of the likelihood of significant clinical impact on the animal, which may continue for a number of weeks, a prospective severity classification of **SEVERE** is deemed appropriate.

Could the severity be MODERATE?

Whether severity could be reduced to moderate depends upon the purpose of the study. For example, with frequent, detailed monitoring of the animals and where there is the potential to implement early end-points (e.g. at onset of lameness, or after a period of mild lameness in one limb, or using *in-vivo* imaging methods to detect early changes in joint pathology), it may be possible to classify the procedure as MODERATE. Such early end-points (e.g. ending the study on day 6 after imaging) may be possible in projects investigating <u>early</u> inflammatory changes. <u>Prophylactic</u> treatment (starting before full development of arthritis) with novel pharmaceutical agents that have strong anti inflammatory effects and subdue the development of full arthritis may also lead to a reduction in severity to **MODERATE**. However, the type of study illustrated here aims to evaluate treatments for fully established arthritis, so the severity classification remains **SEVERE**.

Could the severity be above the upper limit?

According to Directive Article 15(2), '*Member States shall ensure that a procedure is not performed if it involves severe pain, suffering or distress that is likely to be long-lasting and cannot be ameliorated*'. This study has the potential to cause severe suffering over a number of weeks, which should be considered as long-lasting. If severe arthritis was to develop in all four legs and the animals' suffering was not ameliorated, the study would be above the upper limit of severity and it would be necessary to refine it significantly or to invoke the 'safeguard clause' (Directive Article 55) and apply to the Commission for authorisation.

However, in this example there are measures in place to reduce suffering - while taking into account the scientific objective - including refining the composition, the delivery and choice of the adjuvant, only allowing arthritis to develop in the hind legs, providing a comfortable environment and easy

access to food and water, a comprehensive monitoring system and humane endpoints. This project would thus not be considered to be above the upper limit and can be authorised subject to an otherwise positive project evaluation including review of the harms and benefits.

An example of a clinical score sheet for day to day observation of arthritic rats is shown below.

Date:	Day 1	Day 2	Day 3	Day 4
Appearance				
Body weight				
Lack of grooming				
Dehydration				
Body functions				
Dyspnoea				
Tachypnoea				
Behaviour				
Reluctant to move				
Lethargy/apathy				
Immobility				
Vocalization				
Procedure-specific indicator				
Arthritic paw score (see Table 2)				
Other observations				
(Free text)				
Total score				

Note: Each indicator was assessed according to the system in Tables 1 and 2 below. For example, '1' would be entered into the score sheet next to 'lack of grooming' (Table 1), and '5' next to 'procedure-specific indicator' if the two hind limbs scored '3' and '2' respectively (Table 2). The actions and endpoints set out below take account of the requirements to avoid severe suffering wherever possible, but not to humanely kill animals before sufficient data has been obtained, which would make it necessary to use further, naïve animals.

Table 1. Scoring system for indicators used in the clinical score sheet

	Score
Appearance	
Normal < 5% weight loss	0
5-10% weight loss	1
11-15 % weight loss	2
16-20% weight loss	3
20% + weight loss	HEP
Lack of grooming	1
Pinched skin/dehydration	1
Body functions	
Dyspnoea	2
Tachypnoea	1
Behaviour	
Reluctance to move	1
Lethargy/apathy	2
Persistent Immobility < 24h	3
Immobility >24h	HEP
Vocalization on handling	1
Vocalisation, tense and nervous on handling	2
Vocalization on moving/spontaneous	3
Procedure-specific indicator	
Arthritic paw score (Table 2)	0-8

HEP: humane endpoint implemented, regardless of presence or absence of other clinical signs

Table 2. Procedure-specific indicator – arthritic paw score

0	Normal
1	Erythema and swelling of one ankle
2	Erythema and swelling of ankle and proximal half of tarsal joints
3	Erythema and swelling of ankle and all tarsal joints up to metatarsal joints
4	Erythema and swelling of entire paw, including digits

This arthritis scoring system used as a procedure-specific indicator is based on increasing levels of swelling and periarticular erythema. The scores are based on physical examination and visual inspection and are used to calculate an 'arthritic index' which is defined as the sum of the scores for both hind-limbs.

Examples of appropriate interventions in response to total clinical scores

Actions to be taken	Total score
Increase frequency of monitoring; consider supplementary fluids/care	≥4
Review progress with vet	5-15
Humane-endpoint	≥16

Note: The total scores are taken from the clinical score sheets, filled in according to the scoring systems in Tables 1 and 2. For example, an animal with a body weight loss of 12 %, evidence of reduced grooming and swelling in both hind ankles would have a total score of 5.

Retrospective assessment:

36 rats were immunized with bovine collagen Type II in Freund's Incomplete Adjuvant (FIA). All animals developed arthritis: arthritic paw scores were 6 by D10. All animals showed a weight loss of 5-10%. Joint diameter measurements indicated a significant change from baseline data. Daily clinical observations included lack of grooming, reluctance to move, apathy, vocalization on handling (during observation and cage change), decreased food intake and periods of immobility.

- 12 animals were used in the saline treated group. The highest arthritic paw scores were between 6 and 8 for all measurements (D13, D16, D20 and D24). Joint diameter measurements also indicated significant increases vs. baseline at each time point. Clinical scores ranged from 4 to 8, with a body weight loss between 5 and 15%, except in one animal which reached a weight loss of 21% on D17 and was then humanely killed.
  Retrospective assessment: SEVERE

- 12 animals were treated with DRUG A at dose **Low**. In all animals arthritic paw score did not differ from those of the saline-treated group until D16. On D20 one animal had a paw score of 8, the others' scores were 6 to 7.

On D24 five animals showed a somewhat decreased arthritic paw score (5 to 7). Clinical signs of these five animals showed some improvement, body weights were still decreased by 5 to 10%; their mobility in the cage also remained decreased.
The other seven animals did not show reduced arthritic and clinical signs compared to the saline-treated group.
Retrospective assessment: SEVERE

- 12 animals were treated with DRUG A at dose **High**. On D13 arthritic paw scores were between 4 and 6; joint diameters also showed a non-significant decrease. Clinical signs included a lack of grooming and bodyweight loss < 10%. On D16 arthritic paw scores decreased to 4 and joint diameters showed significant reductions. Body weights stabilized at D16. Reluctance to move was still observed in some of the animals. From D20 onward joint swelling was reduced to between 2 and 4. Normal behaviours were observed in the cage. Body weight recovered to pre-procedure levels.
  Retrospective assessment: SEVERE

Note: By the end of the study, in the third group of twelve animals, the test agent 'DRUG A' given at the **High** dose proved effective in reducing the actual severity to Moderate. However, because the model required fully established arthritis to develop in all animals before start of treatment, at which time the animals showed clinical signs consistent with a "severe" classification, the actual severity classification for these animals remained as **Severe**.

As this project involves severe procedures, ongoing reviews of severity are regularly conducted by the user, in discussion with the Animal-Welfare Body, animal technologist and designated veterinarian, to ensure that the 3Rs are continuously applied.

Illustrative examples of the severity process
Model 4 – Stroke

Last updated: 05 February 2013

General context

Stroke is defined as loss or alteration of normal body function that results from an insufficient supply of blood to part of the brain. Despite better understanding of the pathophysiology of vascular brain injury, an effective treatment for stroke remains an important unmet medical need, and research is ongoing to find appropriate preventive and therapeutic measures.

Three different types of stroke can be seen in human patients: ischaemic, intracerebral haemorrhage and subarachnoid haemorrhage, but most of the animal models currently available are based on the ischaemic type. Stroke models, by their very nature, represent a challenge from the perspective of animal welfare. Good interactions and communication between all individuals involved in the scientific procedures, (veterinarians, investigators, animal technologists and care staff), are critical to ensure that there is adequate balance between achieving a valid model in this research area and minimising animal suffering.

Stroke is routinely induced in rodents by temporarily or permanently occluding the middle cerebral artery (Middle Cerebral Artery Occlusion; MCAO model). This 'MCAO' model aims to reproduce experimentally the focal cerebral ischemia that occurs in stroke, and it has been extensively used to study the mechanisms of injury, to identify potential targets and to test putative neuroprotective agents. Strain differences in mice and rats have been identified, as well as the complex and significant influence of age, sex and co-morbidities such as diabetes, hypertension and atherosclerosis. Whereas preclinical stroke research often uses healthy male juvenile rodents, the impact of factors such as those mentioned above can be explored using models with co-morbid conditions (e.g. Spontaneous Hypertensive Rats, Streptozotocin (STZ)-induced diabetes in rats). In such cases with co-morbid conditions, more careful observations of clinical signs and earlier humane end-points (HEP) may be necessary.

In a standard study design, the animals are trained to perform certain behavioural tests prior to the MCAO procedure. During the therapeutic time window, established according to the mechanism of drug action and objective of the study, animals are given the test compound. The outcome analysis should include information on infarct size, mortality rate, frequency of complications (e.g. subarachnoid haemorrhage), together with functional and neurological evaluation to monitor progress. Serial magnetic resonance imaging (MRI) has proven to be a powerful tool to gain information on variation of infarct size over time, but can also provide additional information on blood flow or metabolic state. Histological, biochemical and molecular end-points can also be included.

There are various behavioural tests that may be applied to stroke models. The simplest tests include neurological scoring systems, which assess global neurological status, and limb placing tests, used to measure motor reflexes. These are generally used to assess animals in the acute post-stroke phase. In

long-term studies, more complex tests may be used to assess sensory and motor functions (e.g. bilateral sticky label test, beam walking, rotarod or staircase) and cognitive functions such as memory (e.g. passive avoidance tests, or evaluations of learning strategies).

It is good practice to perform a group of behavioural tests, including at least one for each phase (acute and long-term), so as to gather comprehensive information on the impact on sensory, motor and cognitive functions. These tests have to be carefully chosen to capture any effects of the putative therapeutic strategies. Detailed descriptions of each of these behavioural tests, including training schedules, are not included here, but for a comprehensive review and discussion of their use see Schaar et al. (2010).

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Study

Efficacy of a novel therapeutic agent on intraluminal thread middle cerebral artery occlusion (MCAO) model in rats

In this example, 40 young male Sprague-Dawley rats (300-350g) will undergo permanent MCAO using the intraluminal filament technique under general anaesthesia. Rats will be randomized (n=10/group) to receive either vehicle (10ml/kg) or a new test agent (compound A) at 1, 3 or10 mg/kg, infused intravenously into a tail vein over 1h beginning 30 min post-MCAO. Subsequent doses (either vehicle or compound A at 1, 3 or 10 mg/kg) will be given intra-peritoneally at 6 and 24 h post MCAO. Rats will be initially pair housed in solid floored cages with deep litter and nesting material. Food will be restricted during pre-training to facilitate performance on the staircase test, which was an appetite motivated task. Animals will be provided with food *ad libitum* from 6 hours pre-surgery until 6 days post-MCAO to improve postoperative weight and recovery.

Functional outcome will be assessed daily using a neurological scoring system (the Bederson scale; see Schaar et al. 2010) and behavioural tests (bilateral sticky label test and beam walking); The staircase test will also be performed daily from day 7 post-MCAO, to allow enough time for post-op recovery before food restriction is reintroduced. None of the behavioural tests is expected to cause significant distress. Magnetic Resonance Imaging (MRI) will be performed in anaesthetized rats on days 1, 7, 14 and 28 to assess lesion volume. All animals will be killed 28 days post MCAO.

Initial prospective assessment and consideration of specific refinements and humane end-points

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might it make it worse?**	**How will suffering be reduced to a minimum?**	
	Adverse effects	**Methodology and interventions**	**End-points**
Pre-operative training on behavioural tests over a 2-3 week period: bilateral sticky label test (for contralateral neglect), beam walking (for hindlimb coordination) and staircase test (for skilled forelimb paw-reaching)	Minimal stress/ anxiety can be caused before animals have habituated to the tests, as testing involves moving animals to novel rooms/arenas	Gradual habituation to test apparatus Calm, empathetic handling .	Removal from session if signs of distress observed Animals not reaching a baseline performance within a preset time limit will be excluded from the study
Food restriction (85-90% of free feeding weight) pre-operatively and from 7 days post-MCAO to facilitate performance on staircase test	Mild hunger; possible frustration and anxiety	Weight loss will not exceed 10%, otherwise food restriction will be suspended	If behavioural problems due to lack of food intake are observed, animal will be removed from study
Under general anaesthesia, transient (90 min) occlusion of the MCA using an intraluminal thread advanced via the common carotid artery	Pain and discomfort associated with surgery Potential for unexpected surgical complications, e.g. subarachnoid haemorrhage, ipsilateral retinal injury, intraluminal thrombus formation, brain	Use of appropriate and minimally aversive anaesthesics, with appropriate analgesics (i.e. effective yet with minimal neuroprotective properties) Well-trained surgeon using	Animals will be humanely killed if any of the following occur – • Significant technical problems occur during surgery.

	oedema hypothalamus involvement with consequent hyperthermia or temporal muscle necrosis. These can present in a number of different ways, for example – sudden collapse, paralysis, severe head tilts, seizures Aversiveness and potential effects of anaesthesia on physiological variables (such as hypothermia, hypotension, hypoxia) Poor nutritional intake resulting from reduced consciousness level, impaired mastication and poor motility, generally in the first 48h post MCAO Degree of locomotor deficit, which could cause stress and/or frustration	appropriate aseptic surgical technique (with regular reviews of success rates) Maintenance of homeostasis during anaesthesia Use of standardized monofilaments and surgical technique to reduce variability and complications derived from extensive lesions Intensive post-operative care for first 3-5 days, including external heat sources. Regular body weight checks; daily observation and wound care Providing easy accessible food and water during the recovery period, or additional food (mash, liquid) and assistance with feeding if necessary; rehydrate (e.g. via saline injection) if necessary	• Failing to fully recover from anaesthesia • signs of unexpected surgical complications • If animal's bodyweight loss exceeds 20% pre-surgical weight, despite additional feeding and/or rehydration, or if they remain immobile for over 24 hours
Behavioural tests (bilateral sticky label test and beam walking test) undertaken daily from day 1 to day 28 post-MCAO; staircase test undertaken daily from day 7 post-MCAO	Animals may find the tasks stressful if their motor abilities are compromised	Monitor for behavioural indicators of anxiety or distress Animals will be continuously observed by experienced staff	Typically, a maximum time (cut-off) to perform the requested task is set, and a final score is given
Administration of novel therapeutic agent by s.c/ i.v/ i.p.	Transient discomfort associated with administration route	Administration according to good practice using, with the least	Animals will be humanely killed if any severe side

route before and/or after surgery (prophylactic/therapeutic)	No adverse effect expected at the dose levels administered	painful/distressing route and techniques possible in accordance with scientific objectives. Animals will be closely observed for adverse effects of test substances	effects due to the novel therapeutic agents are noted
Longitudinal MRI under anaesthesia on days 1, 7, 14 and 28 post-MCAO	Repeated anaesthesia Aversiveness and potential effects of anaesthesia on physiological variables (such as hypothermia, hypotension, hypoxia)	Use of appropriate and minimally aversive anaesthesics Maintenance of homeostasis during anaesthesia, including fluid therapy before or during if there are problems with dehydration and heating to maintain normothermia	Animals failing to fully recover from anaesthesia will be euthanased Animals will be humanely killed if homeostasis cannot be maintained following recovery

Analysis

This model is considered to be SEVERE because of the surgical procedure involved, the adverse (but usually transient) effects of the MCAO on the welfare of the animal, and the possibility of significant peri-operative complications. However, the negative impact on animal welfare can be reduced by intensive post-operative care for at least the first 48h, and close monitoring of the subsequent phase - with prompt action taken if there are any problems. From the experimental point of view, attention to refinement and standardization of each of the single procedures can lead to a reduced incidence of complications and variability, and consequently better data quality and a reduction in the number of animals used.

A prospective severity classification of SEVERE is therefore appropriate

Could the severity be MODERATE?

Although prospective severity of this model should always be SEVERE for the reasons outlined above, the incidence of the severe effects can be reduced in the hands of experienced operators, together with expert veterinary supervision and animal care, and agreed early interventions if complications arise. A MODERATE severity could potentially be authorized in some instances, but only on a case by case basis to individual research groups that have a proven track record of experience with this particular model and are known to be able to use the model without causing more than moderate suffering.

Clinical observation

Animals are very carefully monitored in the post-operative period. Analgesia and local supportive therapy are provided as necessary.
An example of a combined neurological/clinical scoring system which is used to help monitor the clinical condition of the animals throughout the procedure is included at the end of this example.

Results

All animals, except one in the vehicle treated group, recovered from surgery with no unexpected complications, due to the intensive peri-operative support provided.

- All 10 Vehicle treated animals showed the lowest neurological score throughout the study, together with a poor performance in the behavioural tests compared to treated animals. Clinical score was similar to treated animals in the immediate (first 48 h) post MCAo, afterwards differences were noted amongst animals in the vehicle group:
  - 1/10 had to be euthanased on day 2 post-surgery due to body weight loss >20% (despite additional feeding and rehydration).
    Assessment: SEVERE
  - 6/10 developed moderate neurological deficit, but showed minimal improvement in clinical score over time
    Assessment: SEVERE
  - 3/10 animals developed moderate neurological deficit, and showed a gradual reduction in clinical score over time, possible resulting from their ability to compensate and adapt to long term neurologic deficits
    Assessment: MODERATE

- All 20 compound A treated animals at lower doses (1 and 3 mg/kg) showed an improvement in neurological scoring after 48h post-MCAO, together with an improvement in clinical scoring.
  Assessment: MODERATE

- All 10 Compound A treated animals at the highest dose (10 mg/ kg) showed improvement of neurological scoring compared with vehicle group from 24h post-MCAO, only minimal (5%) body weight loss 24 hours post-surgery, and significant improvement in clinical scoring from 48h post-MCAO
  Assessment: MODERATE

Assessment of Actual Severity

7 animals were considered as **SEVERE**; 33 animals were considered as **MODERATE**

Scoring system

Severity assessment is performed by a combination of general clinical observations (bodyweight, appearance, behavior, cage environment) together with a procedure specific neurologic evaluation. The Bederson scale is a global neurological assessment that was developed to measure neurological impairments following stroke. A grading scale of 0-3 is used, with 0= normal and 3= highest level of disability. Tests include forelimb flexion, resistance to lateral push and circling behaviour.
grade 0: no observable deficit
grade 1: forelimb flexion
grade 2: decreased resistance to lateral push (and forelimb flexion) without circling
grade 3: same behaviour as grade 2, with circling
HEP: humane endpoint

	Score
Appearance	
5-10% weight loss	1
11-15 % weight loss	2
16-20% weight loss	3
20% + weight loss	HEP
Coat slightly unkempt	1
Slight piloerection	2
Marked piloerection	3
Behaviour	
Slightly abnormal gait	1
Markedly abnormal gait	2
Significant mobility problems	3
Immobility >24h	HEP
Tense and nervous on handling	2
Markedly distressed on handling, e.g. shaking, vocalizing, aggressive	3
Environment	
Slightly disorganised nest	1

Actions	
Score 1	Review frequency of monitoring
4	Provide supplementary care, *e.g.* extra fluids and wet mash
5	Review progress with veterinarian
12	Implement humane endpoint

Nest barely recognisable	2
No nest	**3**
Neurological scoring	
Forelimb flexion	1
Decreased resistance to lateral push(and forelimb flexion) without circling	2
Same behaviour as grade 2, with circling	3

Actions – Note that as surgical complications are generally noted in the immediate post-op recovery period, close monitoring and expert, empathetic judgement are essential during the first 24 hours to ensure that adverse effects are identified and actions taken to address these, and animals are humanely killed if their suffering exceeds the severe category.

Example of an Individual observation sheet (Days 0-4)

Day	**0**	**1**	**2**	**3**	**4**
Appearance					
Body weight (g) (score)	320 (0)	292 (1)	285 (2)	287 (1)	292 (1)
Coat condition					
Coat unkempt/piloerection	1	1	0	1	0
Behaviours					
Gait	3	2	2	2	1
Response to handling	0	0	2	0	0
Environment					
Nest condition	3	2	1	0	0
Procedure-specific neurological scoring	2	2	1	1	1
Total score	9	8	8	5	3
Lesion volume (MRI assessment)*		11 %			
Other observations	Recovered uneventfully from surgery, no complications Dosed at 30 min and 6 h	Moving around cage and has attempted to make a nest	Behavioural tests, anxious at first but all completed, nest more structured	Coat less well groomed today but weight stable and good nest	Behavioural tests completed, less anxious and gait markedly improved

***** 'lesion volume' (assessed using MRI) is included for the investigator to fill in at the end of the study. This data can then be correlated with clinical and behavioural observations to enable further refinement of monitoring, animal care and procedures.

Illustrative examples of the severity process
Model 5 – Production of Polyclonal Antibodies in Rabbit

Last updated: 05 February 2013

General context

The primary goal of antibody production in laboratory animals is to obtain high titre, high affinity antisera for use in experimentation or diagnostic tests. Much of modern biology and biochemistry relies on the availability of highly specific antibodies for use in a variety of techniques such as immunohistochemistry, ELISAs, immunoprecipitation, and immunoblotting. Thus, the generation of large quantities of specific antibodies directed to proteins or peptides of interest is essential to the success of many basic and applied research programs
In this example, a rabbit will be used to raise antibodies to small peptides that are considered to be of importance in the regulation of cell division, as part of a research programme involving biochemical studies of mammalian cell division.

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Study

From previous experience it was determined that a single rabbit should provide sufficient material for each peptide of interest. The rabbit will be housed in a floor pen in a stable group of compatible rabbits (also used for antibody production), provided with adequate space for enrichment, exercise and normal social behaviour (UFAW/RSPCA 2008).The animal will be immunised with an antigen/adjuvant mixture. At predetermined time-points small volumes of blood will be sampled to determine if immunisation has been successful. When a suitable antibody titre has been obtained, the animal will be bled under deep anaesthesia without recovery to collect the antibodies in the blood.
Handling of rabbits can be stressful and should only be performed by competent and empathetic staff. Rabbit behaviour can be difficult to interpret and it is good practice to maintain knowledge of the literature on rabbit behaviour and welfare. For example, recent literature has indicated that 'pain faces' may be displayed by rabbits under certain circumstances (Keating et al. 2012) and the potential to use this as a tool for welfare assessment should be explored on a case by case basis.
Because of the poor immunogenicity of the short chain peptide, it will be necessary to administer it in combination with an adjuvant. Freund's Complete Adjuvant (FCA) has been used previously, but synthetic adjuvants are now available which are also effective for this procedure and minimally irritant.

Initial prospective assessment and consideration of specific refinements and humane end-points

What does this study involve doing to the animals?	**What will the animals experience? How much suffering might it cause? What might it make it worse?**	**How will suffering be reduced to a minimum?**	
	Adverse effects	Methodology and interventions	End-points
Immunisation with antigen and adjuvant; three subcutaneous injections on days 8, 22 and 37	Discomfort following injection Non painful lumps may develop in response to the adjuvant Potential (rare) for ulceration at the injection site	Injection volume, formulation and frequency will be in accordance with good practice guidelines (e.g. EFPIA/ECVAM or JWGR), typically a maximum of four sites and 0.25 ml per site Any ulcers will receive appropriate veterinary treatment immediately	Animal will be humanely killed if there are any signs of prolonged discomfort, pain or distress (e.g. persistent attention to injection sites or lumps), or if ulcers form that do not heal
Blood sampling to assess antibody	Capture, handling and restraint, which	Sampling will be from superficial (ear)	If animal becomes unduly

responses (on up to 5 occasions)	can be stressful. Minor discomfort associated with needle stick Low risk of haemorrhage or haematoma formation	vein. Small volumes of blood (<5ml) only to check antibody titres Apply pressure to sampling site	stressed by the procedure, sampling will be delayed until the animal's behaviour has returned to normal
Exsanguination under general anaesthesia	Minor discomfort and possible aversion to the agent during induction of anaesthesia	Minimally aversive anaesthetic agent used	Animal will remain under anaesthesia until death

Analysis

Only mild severity is expected, due to the refinements in husbandry and care, good practice for administration and sampling, and choice of a minimally irritant adjuvant.

A prospective severity classification of MILD is therefore appropriate

Could the procedure be further refined?

The potential to use minimally irritant adjuvants and less aversive anaesthetic agents should be regularly reviewed, by monitoring the literature and discussing the issue with colleagues. A programme of habituating juvenile rabbits to handling could be set up, to further reduce stress (UFAW/RSPCA, 2008).

Clinical observations

As only minor adverse effects were expected in this study, a basic monitoring system was used; i.e. the animalwas checked daily and observations were recorded, but no structured recording sheet was considered to be necessary.

An illustrative example of an observation sheet is included at the end of this example.

Assessment of actual severity

Some transient, slight swelling at one injection site was recorded but no treatment was required. The rabbit showed some attention to the injection sites for a short duration, but this was believed to indicate mild discomfort only. No 'pain faces' were observed.
No adverse effects were noted due to the actual blood sampling from the ear vein.
An actual severity of **MILD** for this animal was considered appropriate**.**

Example observation sheet

Rabbit Antibody Production – Procedure & Observation Sheet		
Date	Body-weight (kg)	Comments
01/03	3.5	Pre-bleed – 5ml ear vein ; no adverse effects noted
02/03		No Abnormality Detected (NAD)
06/03		NAD
07/03		NAD
08/03	3.6	Immunised – 0.25ml x 2 sites s/c, slight attention to sites (grooming) for several minutes then back to normal
09/03		NAD
10/03		NAD
11/03		NAD
12/03		Slight, soft non-painful swelling at LHS site.
13/03		Still swelling at LHS site, no worse
14/03		Swelling at LHS site still present but not painful on palpation
15/03	3.6	Swelling gone, all normal

21/03		NAD
22/03	3.6	Immunised – 0.25ml x 2 sites s/c, brief attention to sites

28/09		NAD
29/03	3.7	NAD

30/03		Test bleed – 2ml ear vein, no adverse effects

05/04		NAD
06/04	3.6	Immunised – 0.25ml x 2 sites

14/04	3.6	NAD
15/04		Test bleed – 2ml ear vein, no adverse effects

26/04		NAD
27/04	3.6	Exsanguinate under general anaesthesia, no adverse effects

Confirmation should be kept that the animal has been checked at least daily – e.g. on the individual animal record (as above) or on the room record.

Illustrative examples of the severity process
Model 6 – Production and Maintenance of Genetically Altered (GA) Animals

Last updated: 05 February 2013

1. General context

The use of genetically altered (GA) animals in research has contributed to the understanding of the function of genes and their corresponding proteins. Different phenotypes can have a variety of effects on animal welfare, and some can cause pain, suffering or distress. While some phenotypes and outcomes are predictable, many unexpected or secondary traits can occur during the creation of GA lines, so it is not always possible to accurately predict severity. In practice, the phenotype is not affected in many GA lines and assessment protocols can be set up to ensure that any adverse phenotypes can be detected. Alternatively, the expected phenotype can often be associated with unforeseen secondary phenotypes that manifest at different time points and may be affected by different environmental factors.

When assessing the actual harm to the animal, multiple factors should be taken into account such as the type of mutation, genotype, phenotype and breeding strategy (e.g. avoiding harmful homozygous phenotypes by mating heterozygotes x wild type), along with the nature of any additional scientific or husbandry procedures and the potential effects of all of these. Systematic and appropriately timed observations, both during colony progression and throughout the experimental phase of a colony, are necessary for effective assessment of the animal's welfare state.

New lines should be carefully monitored and subject to a standard welfare assessment. All lines should be assessed individually by appropriately trained and competent staff during colony progression and maintenance, and information on specific observed adverse effects should be collated and reported. Licensed personnel should apply any scientific procedures involved and, in conjunction with the care staff, monitor and record any effects on the animals. Humane endpoints should be prospectively set with respect to parameters such as weight loss, body condition and behaviours of concern, along with specific developmental characteristics. No animals should be kept alive if they exceed the predicted severity limit unless they are of compelling scientific interest, and then only with authorisation from the Competent Authority.

The nature, timing and duration of observations will depend upon a number of factors other than the applied mutation. For example, the genetic background and environmental conditions under which the animals are maintained can significantly alter the expression of the phenotype. These specific factors should be accurately noted to facilitate better comparisons between facilities and monitoring of GA animals in general. The lifespan of each line within a particular facility should also be taken into account, as some phenotypes are of a late onset and so will only be observed if animals are kept for longer durations.

References
RSPCA GA Passport Working Group (2010) *GA Passports: The Key to Consistent Animal Care*. Southwater, UK: RSPCA (download at http://www.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/gapassport)
Wells DJ et al (2006) *Assessing the welfare of genetically altered mice Laboratory Animals* **40(2):** 111-114 (download at http://www.nc3rs.org.uk/downloaddoc.asp?id=356&page=231&skin=0)

2 Examples

The three examples in sections 2.1 to 2.3 below illustrate how severity may be assessed in GA mice, including review of developmental milestones, procedural impact and colony development. Each example focuses on the principles of severity assessment rather than taking into account every possible scenario throughout the development of the colony.

The creation of each model will follow standardised procedures requiring surgical preparation of vasectomised stud males, the manipulation of embryos and their surgical implantation into recipient pseudo pregnant females. Good practice is assumed with respect to asepsis, pain management and the competence of the surgeon.

Confirmation of the presence in founder or germ line derived offspring will be ascertained from tissue samples obtained as a by-product of identification (ear notching) or by the least invasive method that supplies sufficient tissue for genotype assessment. The phenotyping strategy for each line will depend upon the gene, research area and predicted effects. Severity assessment will be determined by a series of standardised observations.

2.1 Genetically Altered mouse model – *GeneA*$^{tm1a(Funding)Lab}$

2.1.1 General context

A colony of mice was created with a novel mutation in *GeneA* which was targeted to an Embryonic Stem cell line derived from the C57BL/6N background with an unknown phenotypic potential. The model was maintained in a defined background (C57BL/6N). Once germ line transmission of G1 mice had been established, a basic welfare assessment screen was carried out using 30 pups from 3-5 litters from independent matings. The offspring were monitored at set milestones in the development of the colony - at birth, 14 days after birth (in conjunction with identification of pups and recovery of tissue for genotyping) and at weaning. An appropriate score sheet was developed on the basis of a GA Welfare Assessment Scheme (Wells et al. 2006). Observations of the pups were performed by animal technologists at the cage side, with colony managers monitoring the genotypic ratios. The mice were group housed after weaning where possible, in individually ventilated caging containing litter, nesting material and environmental enrichment as appropriate. Animal technologists carried out cage side assessments during their daily interactions until the mice reached sexual maturity. Longer term assessments for age

related adverse welfare effects were monitored and recorded from stock animals and future breeding stock. Any observations were compared to the background strain and their relevance assessed.

2.1.2 Prospective assessment

What does this study involve doing to the animals?	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects	**Methodology and interventions**	**Endpoints**
Baseline effects of genetic alteration	Genetic modification may lead to clinical adverse effects In cases where these are unpredictable, any indication that animals with the mutation have moved away from normal physical or behavioural parameters (i.e. those that are known occur in genetic background related phenotypes and/or wild type controls) could denote a welfare problem	On-going cage side monitoring Welfare assessment at defined developmental time points; birth, weaning and sexual maturity Depending on the nature of any detected adverse effect, appropriate ameliorating factors will be applied where possible such as altered breeding strategies or husbandry refinements (e.g. increased nesting material to assist impaired thermoregulation	Animals will be killed if moderate severity is exceeded
Tissue sampling for genotyping	Potential pain and/or distress caused by tissue sampling methodology, e.g. ear punching/notching or tail 'tipping' Tail biopsy is commonly used when larger quantities of DNA are required, but may cause both short and long term pain (the latter due to neuroma formation)	Where identifying individual animals using ear notching, it is good practice to use the ear tissue for genotyping where possible For tail 'tipping', the minimum amount of tail should be taken (bearing in mind that repeat sampling is highly undesirable), anaesthesia and analgesia should be used as	Not applicable, as the procedure should be a 'one off' and it is unlikely that pain or distress would be caused to a level where humane killing would be necessary

		appropriate and excessive bleeding should be dealt with promptly Developments in less invasive techniques should be monitored, evaluated locally and implemented wherever feasible	
Phenotyping	Stress induced by handling or application of the phenotypic assay, e.g. stress of being placed into an unfamiliar environment, administration of experimental compounds to induce a response, infection monitoring, anaesthesia and restraint for imaging etc.	Training of staff conducting phenotyping in competent, empathetic and standardised handling and observations Use of anaesthesia during imaging or painful procedures. Structuring of phenotypic tests to move from the least invasive (e.g. observation of behaviour in an open arena), to the most invasive (e.g. procedures requiring anaesthesia)	Where the mutation elicits a severe response to a phenotypic assay, humane endpoints will be reached and animals humanely killed

The gene under investigation is a new mutant with albeit unknown adverse effects. Experience at this establishment has shown that the great majority of similar models generally show a mild phenotype. However, occasionally a model will unexpectedly exhibit moderate clinical signs and therefore, on this basis, this example would be prospectively classified as MODERATE.

2.1.3 Results

Initial assessment in neonatal animals (at birth):

Colour of pups (for neonate only)	Normal
Activity of pups (for neonate only)	Normal
Milk spot	Present

(for neonate only)	
Litter	All pups conformed to the background parameters with respect to litter sizes, litter homogeneity, development and growth of pups

The following indicators were observed at 14 days post birth and weaning:

Overall Appearance	All pups were morphologically 'normal' No indications of malformation were observed
Size, conformation and growth	Normal growth, according to the standard growth curve for the background strain
Coat condition	Normal
Behaviour – posture, gait, activity and interactions with the environment	Normal behaviours and interactions between all cage mates; no hyperactivity or aggression were observed.
Clinical signs	None detected
Relative size	Normal in comparison to the background
Numbers	Pre-weaning mortality rate was normal to the background

Clinical observations

All observations and ratios on neonatal pups to weaning were considered normal in relation to the genetic background (C57BL/6N) with homozygous, heterozygous and wild type mice born at normal Mendelian ratios.

At 4 weeks of age, homozygous and wild type control mice (7+7) were run through a series of observational and mild procedural tests such as SHIRPA, dysmorphology, open field, blood clinical chemistry, DEXA and Faxitron imaging over a 16 week period. At the conclusion of this experiment, phenotypic analysis highlighted a reduction in glucose clearance in homozygous mice after an intraperitoneal Glucose Tolerance Test (ipGTT). Although glucose clearance was reduced during the challenge, post procedure all animals returned to their basal state and no further adverse effects were noted.

2.1.4 Analysis of the results

Actual severity assessment

Following colony establishment, the maintenance and progression of the colony used heterozygous mice and wild type litter mates. No harmful phenotypes were observed in any of the mice used for breeding and maintenance, so these were deemed to show no adverse effects. As no harmful phenotype is expected this line could therefore be made homozygous and maintained without Project Authorisation.

The above mating of heterozygous x heterozygous mice produced homozygous mice. A group of these mice was used for a standard phenotyping screen consisting of a series of mild protocols, including the insertion of a needle for blood sampling during the ip Glucose Tolerance Test. Wild type controls were run through the tests at the same time. The cumulative effect on the mice would have been mild, due to the blood sampling and subsequent phenotyping procedures, as opposed to the overall effect of the genetic alteration.

Summary

Breeding and maintenance – no adverse effects
Homozygous + control mice – MILD – by virtue of the screening tests (not the effect of the genetic alteration)

In summary, this GA mouse line can be considered to have a non-harmful phenotype. Breeding of established lines would not require project authorisation under the Directive.

2.2 Genetically Altered mouse model – *Tg(GeneB)^{Labcode}*

2.2.1 General context

A colony of mice with a mutation overexpressing a transgene will be created as a model to study a form of cancer. The line will be created in a C57BL/6N background. However, the onset and rate of tumour development cannot be defined and will require assessment as part of the model's characterisation. Once founder lines have been established a basic welfare assessment screen will be carried out as described in section 2.1. The most useful line will be progressed to study this type of leukaemia.

2.2.2 Prospective assessment

What does this study involve doing to the animals?	What will the animals experience? How much suffering might it cause? What might make it worse?	How will suffering be reduced to a minimum?	
	Adverse effects	**Methodology and interventions**	**Endpoints**
Assessment and characterisation of tumour development	Weight and condition loss will progress with the development of the cancer	The interventions will be based against daily observations using criteria such as weight loss, loss of body condition, lethargy etc.	Stock and breeding animals displaying clinical signs that are not under experimental procedures such as a weight loss beyond 15%, poor coat condition, lethargy will be humanely killed
	Sub-cutaneous swellings may cause discomfort, affect normal behaviour, posture or locomotion	Daily observations and monitoring of general health and tumour growth	Animals will be humanely killed if the tumour ulcerates, or interferes with the normal behaviour, posture or locomotion, or exceeds 1.2cm in diameter
	Animals may be more susceptible to disease due to a compromised immune system		Animals showing signs of inter-current disease will be humanely killed

The model under investigation will be mutated to create the predicted genetic disorder. The onset of disease cannot easily be predicted but the clinical signs can be predefined to allow onset to be characterised. The model once characterised would need to be maintained to allow its use during subsequent experimental studies on potential treatments for this type of cancer under study. On this basis, this example would be prospectively classified as MODERATE.

2.2.3 Results

Welfare assessments were conducted as in section 2.2 above. No abnormalities in developmental milestones, growth up to sexual maturity were noted. The colony was expanded with stock and future breeding animals mated from 10 weeks of age to maintain the colony and produce new experimental animals. Animals were monitored throughout this time and tumour development was noted from 18 weeks of age in 60% of animals carrying the mutation. The clinical course of the disease was between 4 to 6 weeks at which point animals required euthanasia.

2.2.4 Analysis of the results

Actual severity assessment

Animals that carried the mutation were noted to develop tumours in 60% of the animals from 18 weeks of age. The breeding strategy mated animals from 10 weeks of age. The potential for breeding pairs to develop tumours was considered sufficient to alter the breeding and maintenance. Breeding pairs were then mated from 6 weeks of age and pairings disbanded by 12 weeks of age with stud males killed. Stock and breeding females were monitored daily to detect the early signs of tumour development. Animals that were not used or required were killed humanely before the onset of any clinical signs.

Summary

Animals below 18 weeks of age – no adverse effects
Animals from 18 weeks of age developing tumours – MILD due to early clinical endpoints
Animals from 18 weeks of age developing tumours and issued for use – MILD or MODERATE dependent on the application of clinical endpoints.

2.3 Genetically Altered mouse model – *GeneC*$^{tm1a(Funding)Lab}$

2.3.1 General context

A colony of mice with a mutation in *GeneC* targeted to an Embryonic Stem cell line derived from the C57BL/6N background with a known phenotypic potential was created to test behaviour and memory. The model was maintained on a defined background (C57BL/6N). Once germ line transmission of G1 mice had been established a basic welfare assessment screen was carried out.

2.3.2 Prospective assessment

As in section 2.1, the gene under investigation is a new mutant. The intention is to use the model in future behavioural studies testing the efficacy of novel pharmaceutical compounds. Experience at this establishment has shown that the great majority of similar models generally show a mild phenotype. However, occasionally a model will unexpectedly exhibit moderate clinical signs and therefore, on this basis, this example would be prospectively classified as MODERATE.

2.3.3 Results

All observations and ratios were considered normal in relation to this model's genetic background (C57BL/6N) with homozygous, heterozygous and wild type mice born at normal Mendelian ratios.

At 4 weeks of age, homozygous and wild type control mice were run through a series of observations and tested to assess learning and memory. These tests were conducted over a 10 week period. At the conclusion of this phenotypic analysis no harmful phenotypes were observed. The model was then used to test the efficacy of novel pharmaceutical compounds.

The breeding of the heterozygous mice produced healthy homozygous animals that displayed a similar reproductive performance to the background strain. As such to reduce animal numbers a breeding strategy of homozygous matings was used. In contrast to the original mating, where homozygous mice were derived from a heterozygous x heterozygous mating, the new group of homozygous animals derived from a homozygous parental mating appeared runted and failed to fully regain their size and weight in comparison to their siblings.

Although the line was originally intended as a behaviour and memory model, further analysis was carried out on tissue and blood obtained from these animals. During the analysis of the blood biochemistry results and subsequent literature review, *GeneC* was found to be an essential transporter protein that binds to vitamin B12. The deletion of *GeneC* resulted in a break in the extracellular transport mechanism leading to impairments in DNA synthesis and the metabolism of fat and protein. The effect of this mutation would not have been seen in mice born from a heterozygous female as the maternal vitamin B12 source is transferred in-utero via the placenta to the developing fetus. The original knockouts for this gene therefore had sufficient B12 stored to allow them to survive and thrive to at least 16 weeks of age, ensuring normal breeding and fertility as compared to the background strain.

2.3.4 Analysis of the results

Actual severity assessment
This example demonstrates that colony maintenance can have a profound and often unexpected effect on the mice. On the previously available information and results of the primary breeding and phenotyping, this colony would have appeared unremarkable. Logically, maintaining a colony in a homozygous mating strategy would normally ensure that the minimal numbers of animals were produced, which is desirable in order to minimise animal usage. Unforeseen harmful phenotypes can occur in lines previously maintained as normal animals without project authorisation. As a consequence of the adverse welfare effects on the animals in this example, this model would need to be brought back under project authorisation if this type of breeding scheme was applied.

Summary

Breeding and maintenance of heterozygous pairings – No adverse effects
Breeding and maintenance of homozygous pairings – MODERATE severity for offspring of this generation, due to runting and failure to thrive

Example 2.3 - This highlights the need for the transfer of accurate and useful welfare data between institutes when detrimental phenotypes may manifest themselves, for example in the form of a 'mouse passport'[i].

[i] RSPCA GA Passport Working Group (2010) *GA Passports: The Key to Consistent Animal Care*. Southwater, UK: RSPCA (download at http://www.rspca.org.uk/sciencegroup/researchanimals/implementing3rs/gapassport)

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