CODE OF PRACTICE

ANIMAL EXPERIMENTS IN CANCER RESEARCH

Inspectie W&V
[Netherlands Inspectorate for Health Protection, Commodities and Veterinary Public Health]
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CONTENTS

1. INTRODUCTION

Every year, some 100,000 animals are used for cancer research in the Netherlands. This is approximately 13% of the annual total number of laboratory animals used. The percentage is substantially higher in certain institutes, varying from 25% to even 100%. The vast majority of these animals are mice and rats. In 63% of cases, they involve a moderate to serious degree of distress (Zo doende 1994, 1995, 1996, 1997). There are three categories of cancer research requiring animal experimentation.

1. Basic research: research into basic scientific questions. This often involves mechanistic research.
2. Clinical research: research into the therapeutic effects of possible clinical therapies (cytostatics/radiation/immunotherapy).
3. Safety research: research into the possible carcinogenic effect of substances as part of risk assessment and standard setting.

– An inventory by the Inspectorate in 1995 into the nature and extent of animal experiments in cancer research showed that institutes use different criteria in relation to the following aspects:
– maximum acceptable tumour mass
– endpoint of the animal experiment
– pain relief
– observation of the animals.

During the past 10 years, the Veterinary Inspectorate has devised 4 Codes of Practice with regard to other research fields. In view of the above, the Inspectorate decided to investigate the need for a Code of Practice in relation to animal experiments in cancer research and, if necessary, pinpoint the aspects that needed to be included in a Code of Practice of this kind. A Code of Practice comprises guidelines approved by experts which should lead to responsible animal experiments (in terms of both science and the welfare of the animals concerned) within a particular research field. Codes of this kind can be an important instrument for researchers, laboratory animal experts, members of Committees on Animal Experimentation, people handling laboratory animals and Inspectorate staff.
The United Kingdom (UK) already had 10 years experience of working with a Code for cancer research: the United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) brought out the Guidelines for the Welfare of Animals in Experimental Neoplasia in 1988. These guidelines were widely accepted in the UK, and other countries also started using them. The Inspectorate in the UK used the guidelines for assessing research plans, and in 1997 an up-to-date version of the document was published (Workman et al., 1998).

It is essential to involve experts from the relevant research fields in compiling a Code of Practice of this kind. A Code of Practice is a reflection of generally accepted expert insight into a certain area and therefore by definition a product of its time. As time passes, new scientific developments may mean that a Code of Practice will need to be amended.

<u>The Working Party on Animal Experimentation in Cancer Research</u>
In March 1998, the Veterinary Chief Inspectorate set up the Working Party on Animal Experimentation in Cancer Research. This working party was commissioned to devise a Code of Practice relating to animal experiments in cancer research. Although the working party has now completed its original task of producing this Code of Practice, it will not be disbanded but will continue to function as a group that can be consulted by the Inspectorate as well as researchers, laboratory animal experts and Committees on Animal Experimentation.

The Working Party on Animal Experimentation in Cancer Research is made up of the following members:
- Dr R. ten Berg, Netherlands Cancer Institute, Amsterdam
- R.M.P. Brandt, Leiden University Medical Center, Immunohaematology Department & Blood Bank, Tumour Immunology Section, Leiden
- Dr G.J. Peters, VU University Medical Center, Medical Oncology Department, Amsterdam
- Dr G.J. van Steenbrugge, Erasmus University Rotterdam, Experimental Urology Department, Rotterdam
- P. Dortant, Netherlands National Institute for Public Health and the Environment, Laboratory for Pathology and Immunology, Bilthoven

- W.A. de Leeuw, Health Protection Inspectorate, Commodities and Veterinary Public Health Affairs, Veterinary Products Signalling Department, Zutphen.

Code of practice for animal experimentation in cancer research

The Code comprises a number of basic principles, which have been approved by experts and must be observed when carrying out animal experiments whereby some or all of the animals concerned will develop tumours. This can involve animal models whereby tumours will develop spontaneously (including transgenic and knockout animals), and animal models whereby tumours are induced via, for example, transplantation, radiation or the administration of carcinogenic substances. The Code is obviously not exhaustive and situations may arise in which the Code cannot be followed to the letter. However, any deviations from the Code must be explicitly mentioned and explained in detail in the research plan put before the Committee on Animal Experimentation for approval.

The Code contains a number of guidelines, categorized according to areas of specific attention. A few explanatory chapters and appendices take a closer look at recognizing signs of distress, responsible endpoints and monitoring the animals. Finally, a list of recommended literature has been included.

If you wish to respond to the *Code* or would like to consult the Working Party on Animal Experimentation in Cancer Research, please contact the Inspectorate. The contact address is:
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2. CODE OF PRACTICE

General considerations

* The researcher setting up the animal experiment must satisfy himself in advance that it would not be possible to achieve the required goal by means of an animal experiment whereby fewer animals could be used or less distress caused (Experiments on Animals Act [Wet op de dierproeven], Article 10). The research plan must show that no alternative is available.

* *In vivo*[1] research into the therapeutic effects of substances may only be carried out if prior *in vitro*[2] or *ex vivo*[3] experiments have proved that these substances have potential biological effects. Deviations from this rule must be explicitly stated and explained in detail in the research plan.

Responsible endpoints

* 'Spontaneous' death or higher degrees of distress than expected may not be used as the intended endpoint or a parameter. If the experiment warrants an endpoint of this kind, the research plan submitted to the Committee on Animal Experimentation must contain explicit arguments backing this up.

* Euthanasia may not be put off until an animal is already on the point of dying.

* An animal should be euthanased if it is experiencing more distress than strictly necessary for the aim of the experiment and the distress cannot be relieved in any other way. In the case of cancer research, this means that an animal must be humanely euthanased if one or more of the following points apply:
  1. The animal is no longer eating or drinking.
  2. The animal has lost more than 15% of its bodyweight in a relatively short space of time (1-2 days), or its bodyweight has dropped by more than 20% in relation to its weight at the start of the experiment.
  3. If serious circulatory or respiratory problems arise. The following symptoms may occur: increased breathing or heart rate, laboured breathing, cold and blue extremities or ears, systemic oedema.
  4. The behaviour and locomotion of the animal become seriously abnormal. The following symptoms may occur: passive or hyperactive behaviour, auto-mutilation (self-harming), abnormal muscle tone including cramps.
  5. The tumour causes serious clinical symptoms due to, for example, the location, invasive growth or ulceration.[4]
  6. The tumour mass becomes too big. The maximum acceptable tumour mass in mice is 10% of the normal bodyweight or 2 cm^3. For rats, the maximum acceptable tumour mass is 10%, ± 40 cm^3 or a diameter of 4.2 cm.
  7. If other parameters clearly show that the animal will soon die.
  8. The endpoint of the experiment has been reached. Animals may be used again if their condition allows it and as long as this remains within the framework of the Experiments on Animals Act.

[1] in vivo: research on a live animal; within the terms of the Experiments on Animals Act, this is a live vertebrate
[2] in vitro: research on live tissue or a cell line that has been cultivated outside a living organism
[3] ex vivo: research on live tissue shortly after it has been isolated from a living organism
[4] ulceration: a wound that does not heal

Monitoring/control

* A working protocol (see appendix 1) per experiment must be available for all staff involved in carrying out every experiment involving tumour-bearing animals. This working protocol must at least state: the critical points in the experiment, the expected signs of distress, responsible endpoints and the individual staff responsibilities in relation to the experiment.

* The way in which the animals will be monitored and the method for keeping well-organized records of the results of inspections. The information gathered must be accessible to the researcher(s) responsible for the experiment, other staff involved, the laboratory animal experts and the Inspectorate at all times.

3. DISTRESS

Distress
In order to determine responsible endpoints, one must be aware of the general signs of distress. Distress should be taken to mean stress, discomfort, agitation, fear and pain. These symptoms are accompanied by changes in the physiology and biochemistry of the animal and can lead to pathological changes. There are no objective criteria for measuring the severity of distress. It is always a subjective assessment made by one or more people. Assessing the level of distress will always be a result of continued exchanges of opinions between the researcher, the laboratory animal expert and bio-technician, particularly in the case of new experimental situations. Appropriate measures will have to be taken on the basis of consultation.

Signs of distress
With respect to signs of distress, a distinction can be made between general key signs of moderate to serious distress on the one hand, and species-specific and model-specific signs on the other.

Examples of general key signs of distress include:
- altered eating and drinking habits
- abnormal growth or drop in bodyweight
- abnormal body temperature
- altered consistency, colour or amount of faeces
- lack of inquisitive behaviour and isolation
- abnormal posture and locomotion
- altered depth and frequency of breathing
- abnormal reactions to external stimuli.

In its final report (VHI, 1993), the *Working Party on Scoring Distress* in Laboratory Animals describes key signs specific to animals including mice and rats (appendix 2). In view of the fact that cancer research with laboratory animals in the Netherlands is mainly carried out using mice and rats, the appendices focus exclusively on these two species. The final report of the *FELASA Working Group on Pain and Distress* (Baumans et al., 1994) contains a useful table, which cites 3 categories of distress for 13 key signs (appendix 3). This table relates to rodents and rabbits. Another practical example of a table is that of Jones (1998). He cites 3 categories of distress for 10 key signs. This approach has been developed for mice and rats (appendix 4).
Tables of this kind can be used to compile a score sheet. Particular scores can be assigned to the various levels of distress. Although devising a table for general use might appear difficult, it is possible to gear general tables towards specific types of experiments. In practice, a critical limit can be linked to the total score on the sheet. If this limit is exceeded, the animal should be humanely euthanased. An example of an experiment-specific scoring system is included in appendix 5.

For more information about symptoms and scoring distress, please refer to the section ‘Recommended literature’.

Methods of reducing distress
The distress caused to animals during cancer research mainly involves discomfort (e.g. abnormal locomotion due to the location of the tumour), fear (e.g. on approaching, controlling and handling the animal), general malaise and possibly pain, inflammation, ischemia, diarrhoea/constipation or loss of functions.

Distress can be reduced in several ways:

a. By applying the correct experimental techniques, and providing customized care and accommodation. These aspects can have a huge impact on the severity of distress. Consider, for example, the choice of the right method and location when transplanting or inducing tumours, good clinical inspection, maintaining good access to water, food and resting place, proper care of the animals, etc. These are conditions that relate to starting animal experimental work.

b. Pain relief.

A distinction must be made between post-operative pain, directly related to a surgical procedure, and pain as a result of tumour growth. As post-operative pain can have a serious adverse effect on the physiology of the animal and therefore on the experiment, it would be safe to say that pain relief is often necessary. The need for pain relief in these circumstances can stretch from a period of hours to days after the operation.

If the pain is not directly related to a surgical procedure, the following questions arise:

- is the animal experiencing pain from the tumour(s)?
- is the animal responding to pain relief and when should medication be started?
- how does pain relief affect the experiment?

If the research really warrants the animal being kept alive, refining the experiment by using pain relief (particularly in model studies and therapeutic experiments) might be a possibility. The most common pain relief drugs are non-steroid anti-inflammatory drugs (NSAIDs) and opiates. Both groups of drugs have their own working mechanisms and side-effects (Liles & Flecknell, 1992; Flecknell, 1997). These factors determine whether, and if so which, drugs and dosage can be administered. Drugs can be given systemically or locally. Administration via the food or drinking water is often the cheapest and simplest method, and is also suitable for opiates. However, it should be noted that animals in an experiment situation may eat or drink less than usual, and possibly not at all.

Using pain relief does not necessarily reduce the severity of distress in animals with tumours (Van Loo et al., 1997). It is a fact that 20% of cancer patients suffering pain do not experience relief after using conventional analgesics (Hanks and Forbes, 1997). The use of analgesics sometimes merely postpones the distress. The aim of the research can sometimes justify this.

Pain can have negative effect on the immune system, thereby accelerating the growth rate of the tumour. Thus pain relief may influence the results of the experiment.

To sum up; although good remedies are available for post-operative pain relief in animals, chronic pain relief for laboratory animals with tumours cannot always be given effectively. If pain relief is not given, the reasons for not administering pain-killing drugs must be explicitly explained in the research plan.

c. Euthanasia.

Euthanasia is another way of ending distress. Obviously the methods used must be approved by experts and must take account of the type of animal and the circumstances (Close et al., 1996 and 1997).

4. RESPONSIBLE ENDPOINTS

The commonly-used term 'humane endpoints' is fairly controversial and seen by many, including members of the working party, as a euphemism. This document therefore uses the term 'responsible endpoints'.

Definition
Responsible endpoints are the chosen endpoints in the life of a laboratory animal, whereby although animals undergo distress, a conscious decision is made to limit the severity of the distress. The endpoint can be chosen so that the sought-after information can be obtained without long-term serious distress or without entering the moribund (dying) phase. An endpoint can also be decided upon if an animal is expected to suffer long-term distress after the end of the animal experiment (refer to: Experiments on Animals Act , Article 13.4).

Reasons for the choice of responsible endpoints
One of the general principles when carrying out experiments on animals is that although the animals will inevitably undergo distress, the severity of the distress should be consciously restricted. Implementing responsible endpoints is a way of upholding this principle. The choice of responsible endpoints is based on the assumption that animals which die during an animal experiment suffer, and that the level of suffering increases in line with the proximity of death. These assumptions are often justified, but probably not always correct.

When determining responsible endpoints, one should be aware of the general (clinical) signs of distress. One must also recognise the clinical, biochemical and physiological parameters that herald the approaching moribund phase. Consideration must obviously be given to the possible extra distress caused by procedures necessary for evaluating these parameters on the one hand, and on the other hand, the level of distress that the animal could be saved on the basis of the findings of this evaluation.

The decision on the exact moment at which an animal should be euthanased depends on the species of animal, the severity of the distress, the aim of the experiment and any legal stipulations. If, for example, a control animal in a carcinogenicity study develops a perceptible tumour that is not causing serious distress, the animal will not be euthanased immediately because a 50% survival rate after two years amongst the control group is required to prevent the study from being deemed inadequate on the grounds of too low a survival rate. However, if an animal in a study is diagnosed with an acute, sub-acute or semi-chronic tumour, the decision to euthanase the animal in question will be made sooner. In all cases, where allowing an animal with a tumour to live does not serve a justified purpose, the animal should be humanely euthanased.
Generally speaking, an animal that dies ‘spontaneously’ provides less biologically relevant information than an animal that is euthanased before it reaches the stage at which it becomes moribund. The samples taken from animals that have been euthanased are usually in a better condition and can often be more easily processed. Parameters other than death can provide equally useful information and lead to substantially less distress for the animals. Examples include the interval between the first treatment with a possible carcinogenic substance and first clinical observation of tumour growth, the effect of anti-tumour treatment on the size of an externally perceptible tumour, histopathological analysis of invasion and/or metastasis forming, clinical-chemical and haematological stipulations, such as the number of blast cells in leukaemia-models.
This is why the death of an animal should not be seen as the intended endpoint or the most important parameter in an animal experiment relating to cancer research.

Choice of endpoint

The basic principle is that an animal should be euthanased if it is undergoing or is likely to undergo more distress than is strictly necessary for the aim of the experiment, and if the distress cannot be sufficiently restricted. On this basis, it can be assumed that an animal must be euthanased in one or several of the following situations:

1. A serious deterioration in the animal's condition.
   A loss of 15% of bodyweight in a relatively short period of 1-2 days is deemed to be serious distress. Keeping laboratory animals alive at this stage also provides fewer biologically relevant data.
2. The total tumour mass has become too large.
   The criteria for 'too large' depend on the nature of the tumour, but it is generally accepted that the mass, location, invasion or ulcerative[5] growth of tumours should not be allowed to seriously hamper the normal behaviour of the animals or have serious pathophysiological implications for them. Ulceration can be observed in subcutaneously transplanted tumours as an open wound. This constitutes serious distress by definition. However, not all open wounds are ulcerations. Proper observation is always essential! In the case of an open wound that does not heal promptly, the animal should be euthanased.
   In general, a tumour in a mouse should not grow larger than 2 cm^3. The maximum acceptable tumour mass for a rat is 10%, ± 40 cm^3 or 4.2 cm in diameter. However, if cachexia[6] is observed, the animal must be euthanased sooner.
3. The presence of serious clinical symptoms of another nature.
4. It is highly probable, on the basis of the above-mentioned situations or on the basis of other parameters, that the animal will soon die.

The application of the correct statistical methods plays an important part in choosing and using responsible endpoints. The choice of the right statistical method depends on the type of experiment. The evaluation of an anti-tumour effect requires a different approach from research analysing the biological implications of transgenicity. The use of non-parametric tests is recommended in many cases. When setting up an animal experiment, the researcher should decide on the exact 'power'[7] needed to test the hypothesis sufficiently (Beynen and Montfort, 1995). Standard tables in the manuals can be used. A statistician must be consulted if in doubt.

Alongside good statistical arguments, the research plan should also comprise a clear description of the scientific aims. The Committee on Animal Experimentation can only make its recommendation after a scientific evaluation has been made according to institutional guidelines.

5 ulceration: a wound that does not heal

6 cachexia: rapidly deteriorating physical condition, including loss of weight, muscle atrophy and general weakness

7 power: power of discernment

5. MONITORING THE ANIMALS

The following basic principles are important in terms of monitoring the animals:
1. All animals present in a research institute must be checked at least once a day. This is a routine inspection for emergencies whereby the animals might need extra attention.
2. All animals should be checked individually at least once a week.
3. Depending on the nature of the experiment, extra inspections may be necessary.

The frequency and method of these extra inspections depend on:
- the biological properties of the tumour under given circumstances
- the induction substances and methods used
- effects of other (biotechnical) procedures
- changes in the clinical condition of the animal.

This means that it may become necessary to alter the frequency or method of checking the animals during a particular phase of the experiment.

Researchers have prime responsibility for the experiment. They must convey the relevant and available knowledge on the tumours, substances and bio-techniques used, written (as part of the research plan) in language that can be easily understood, and where possible verbally, to the bio-technicians, animal care takers and other staff working on the research project. For every experiment involving tumour-bearing animals, a working protocol must be available to all staff involved in carrying out the procedures. This working protocol must always state the critical moments in the experiment, the methods for checking the animals, the expected specific signs of distress, criteria for responsible endpoints and the individual personal responsibilities in relation to the experiment and the accessibility of the researcher(s) concerned. This working protocol (appendix 1) must be available in the animal rooms while the inspections are taking place. The results of inspections and any additional actions (examination or consultation) must be recorded. The records must be organized in a way that provides institute staff and Inspectorate staff with easy access to the relevant information.

All routine inspections must be carried out by experienced animal care takers or bio-technicians. The additional inspection must be carried in a way that:
- allows symptoms indicating (imminent) serious distress to be noticed in time detects;
- animals that should be euthanased on the same day or that need close monitoring or extra consultation for the next 24 hours.

This will usually mean that the additional inspection will be an individual control.

Any extra observation or examination should preferably be carried out together with the researcher. Additional inspections should be carried out at least once a day in the case of expected serious distress (fast or invasive (infiltrating) growing tumours and increase in tumour mass) or significant (estimated decrease > 30%) changes in food and/or water intake.

Depending on the type of research, various parameters are assessed during the extra controls. If the findings are translated into a numerical scoring system, it will be easier to ascertain the moment at which the researcher should be consulted or the animal euthanased. An example can be found in appendix 5. It is important to realise that a specific score sheet is needed for each separate experiment.

6. ANIMAL MODELS

Numerous animal models have been devised for the purposes of animal experiments in cancer research. It would not be feasible to summarize existing models in the Code of Practice and the explanatory notes, or to explore the relative suitability of particular models for answering specific research questions. The section entitled ‘Recommended literature’ includes a number of references that may prove useful when evaluating or choosing animal models.

7. RECOMMENDED LITERATURE

Distress

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General

VHI (Veterinaire Hoofdinspectie) (1994; 1995; 1996; 1997). Zo doende. Annual report issues by the experiments on animals section of the Netherlands Veterinary Chief Inspectorate in Rijswijk.

Relevant web and Internet sites:

Product safety assessment, via product info at: www.criver.com/

Whole mouse catalogue: www.rodentia.com/wmc/

Animals for research: www.nci.nih.gov/hpage/extra.html => developmental program => animals for research

Appendix 1: Model of a working protocol

Animal Experimental Procedures code:

Research plan number (Committee on Animal Experimentation):

Name of researcher in charge:

VROM licence number:

LNV licence number:

Title of experiment:

Start date / (estimated) finish date of experiment:

Strain(s) (constructs), number of animals:

Brief description of the aim and nature of the procedures:

Expected clinical symptoms (time of occurrence):

Inspection frequency for the animals:

People carrying out the procedures (tel. no.):

What to do in the event of serious illness/ dead animals:

Use of hazardous substances / safety precautions:

Estimated distress	Number of animals					
	1*	2	3	4	5	6
Slight frequent/long-term						
Moderate frequent/long-term						
Serious frequent/long-term						

* refers to the numbers of the specific procedures specified in the brief description of the experiment

Appendix 2: Key signs of distress in mice and rats

MICE

Laboratory mice have been selected for hundreds of generations on the basis of a variety of characteristics. This has led to significant strain differences in terms of behaviour and reaction to stressors.

APPEARANCE	Eyelids fully or half-closed. Eyes appear sunken, ocular discharge as the condition deteriorates. Piloerection, coat dull and in a poor condition, weight loss, dehydration, atrophy of back muscles. Sunken abdomen: indicates reduced food intake.
FAECES	Defecates and urinates in direct response to stress. Diarrhoea can lead to soiled coat.
BEHAVIOUR	Mice become increasingly aggressive and may bite as the pain or stress increase. Fighting back, biting, trying to bite the cause of the pain or the affected area, increased activity. As the condition deteriorates, the animal becomes subdued or apathetic and isolates itself from the group. Eventually stops responding to surroundings. Stops normal eating and drinking, stops sleeping, stops grooming and scratches a lot. Winces with abdominal pain. May damage affected body part. Behaves in a stereotypical fashion.
POSTURE	Gradually adopts a hunched sleeping position and sits in a dark corner.
LOCOMOTION	Runs suddenly, attempts to escape, finds it difficult to maintain body position. Walks unsteadily.
VOCALIZATION	Squeaks when in slight pain, but this disappears when the pain affects its ability to respond.
PHYSIOLOGY	Fast shallow breathing, whereby nasal discharge sometimes results in a sniffing sound when exhaling. Hypothermia if the condition deteriorates seriously.

Foremost signs of distress: weight loss, biting, piloerection, hunched back, sunken eyes and abdomen, dehydration and diminished reflexes.

RATS

Rats are generally tame and less aggressive towards their own sort and people than mice. However, they can become aggressive and unmanageable when experiencing repeated pain or stressful procedures.

APPEARANCE	Piloerection and dull, unkempt coat. Occasional hair loss. Poor skin tension, signs of atrophied muscles on the back, dehydration and weight loss. Eyelids half or fully closed, eyes appear sunken, ocular discharge occurs frequently and can eventually lead to red rings around the eyes.
FAECES	Defecation and urination can occur as direct response to fear, less evident in the event of pain and long-term stress. Constipation or diarrhoea sometimes occur.
BEHAVIOUR	Animals are initially more alert and aggressive, but later become passive and unresponsive. Increasing sleep disturbance. Stops eating and drinking. Less inquisitive behaviour. Will sometimes damage affected body parts.
POSTURE	Gradually becomes more hunched, often rolled up with the head pressed against the abdomen. Hard, tense abdomen if in abdominal pain. Skews head with earache, for example.
LOCOMOTION	Walking on extended legs is a sign of abdominal pain.
VOCALIZATION	Initially squeaks or squeals, especially when being handled, gradual reduction in noises if the pain or distress continues, unless a sudden painful stimulus is given.
PHYSIOLOGY	Increased breathing rate. Hypothermia is an indication of serious deterioration, a pale appearance can indicate anaemia or blood loss.

Foremost signs of distress: squealing, resisting, fighting back, weight loss, piloerection, hunched posture, hypothermia.

Appendix 3: Key signs classified according to severity of distress (FELASA Working Group on Pain and Distress, 1994)

Mild	Moderate	Substantial
Reduced weight gain	Weight loss of up to 20%	Weight loss greater than 25%
Food and water consumption 40-75% of normal for 72 h	Food and water consumption less than 40% of normal for 72 h	Food and water consumption less than 40% for 7 days, or anorexia (total inappetenceh) for 72 h
Partial piloerection	Staring coatXmarked piloerection	Staring coatXmarked piloerection-with other signs of dehydration such as skin tenting
Subdued but responsive, animal shows normal provoked patterns of behaviour	Subdued animal shows subdued behaviour patterns even when provoked	Unresponsive to extraneous activity and provocation
Interacts with peers	Little peer interaction	
Hunched transiently especially after dosing	Hunched intermittently	Hunched persistently ('frozen')
Transient vocalization provoked	IntermittentXvocalization when unprovoked	'Distressed'-vocalization
Oculo-nasal discharge transient (typically signs of chromorhino-dacryorrhoea in rodents)	Oculo-nasal dischargeXpersistent	Oculo-nasal dischargeXpersistent and copious
Normal respiration	Intermittent abnormal breathing	Laboured respiration
Transient tremors	Intermittent tremors	Persistent tremors
No convulsions	Intermittent convulsions	Persistent convulsions
No prostration	Transient prostration (< 1h)	Prolonged prostration (> 1h)
No self-mutilation	No self-mutilation	Self-mutilation

Appendix 4a: Guidance on Severity Limits - Mouse (Jones, 1998)

		Mild	Moderate	Substantial
Body Weight	Body Weight	Up to 10% weight loss	10% - 20 % Weight loss	>20% Weight loss
Appearance	Posture	Transiently hunched-especially after dosing	Intermittently hunched	Persistently hunched
	Piloerection	Partial	Marked-staring coat	Marked-staring coat + additional signs of unched posture, unresponsive behaviour
Clinical signs	Respiration	Normal	Intermittent abnormal pattern	Persistently laboured
	Salivation	Transient	Intermittent with wetting of fur under chin	Persistent, permanently wet fur
	Tremors	Transient	Intermittent	Continuous
	Convulsions	None	Intermittent (but <10 mins duration)	Continuous (euthanize if > 10 mins duration)
	Prostration	None	Transient < lhr	Persistent> lhr (euthanize if 3 hour duration)
Unprovoked behaviour	Socialisation	Peer interaction	Little peer interaction	No peer interaction
Response to stimulus	Provoked behaviour	Minimal charges	Subdued – responds when stimulated (eg handling)	Unresponsive to extraneous activity or provocation

Appendix 4b: Guidance on Severity Limits - Rat (Jones, 1998)

		Mild	Moderate	Substantial
Body Weight	. Body Weight	. Up to 10% weight loss	. 10% - 25 % Weight loss	. >25% Weight loss
(excluding transient weight loss)	. Food/water consumption	. 40-75% for 72 hrs.	. <40% for 72 hrs.	. <40% for 7 days or anorexia >72hrs
Appearance	. Posture	. Transiently hunched-especially after dosing	. Intermittently hunched	. Persistently hunched
	. Piloerection	. Partial	. Marked-staring coat	. Marked-staring coat + additional signs of hunched posture, unresponsive behaviour
Clinical signs	. Respiration	. Normal	. Intermittent abnormal pattern	. Persistently laboured
	. Salivation	. Transient	. Intermittent with wetting of fur under chin	. Persistent, permanently wet fur
	. Tremors	. Transient	. Intermittent	. Continuous
	. Convulsions	. None	. Intermittent (but <10 mins duration)	. Continuous (euthanize if > 10 mins duration)
	. Prostration	. None	. Transient < lhr	. Persistent> lhr (euthanize if > 3 hour duration)
Unprovoked behaviour	. Socialisation	. Peer interaction	. Little peer interaction	. No peer interaction
Response to stimulus	. Provoked behaviour	. Minimal charges	. Subdued - responds when stimulated (eg handling)	. Unresponsive to extraneous activity or provocation

Appendix 5: Example of an experiment-specific score sheet

The example below is derived from the report entitled 'Guidelines on choosing an appropriate endpoint in animal experiments' issued by the Canadian Council on Animal Care (CCAC, 1998).

It concerns a colon tumour model for rats. The colon tumours are induced using azoxymethane. For the first four months, the animals are monitored according to standard routine procedure. After the first four months, they are examined three times a week. These examinations involve:

- palpation of the abdomen
- examination of the eyes to check for anaemia
- test of faeces for the presence of blood
- assessment of general appearance and behaviour.

The following scoring system is used.

1. Changes in bodyweight

score	findings
0	normal
1	< 10 % loss
2	10-15 % loss
3	> 20 % loss

2 Symptoms

score	findings
0	no abnormalities
1	pale eyes: anaemia
2	anaemia, blood in faeces, diarrhoea/small stools
3	above-mentioned symptoms + increased abdominal volume
4	abnormal posture, lack of hygiene, dull/rough coat

Endpoint

* Humane euthanasation if the weight loss exceeds 15% in comparison with the control animals.
  If the experiment does not allow for euthanasation, pain relief must be used until the end of the experiment.
* Animals with a total score of > 4 must be euthanased.