Monitoring, Clinical Scoring, and Intervention Points Guidelines for Flinders University Bio-Medical Rat and Mice Studies

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October 22, 2020

CONTENTS

1. Executive Summary

The guidelines described in this paper provide a rationale for clinical criteria, monitoring, intervention, and humane endpoints for animals participating in research. The recommendations in this paper are a guide only, as each project should be assessed on an individual basis and the management of animal welfare must be consistent with the *Australian Code for the Care and Use of Animals for Scientific Purposes.*

Monitoring of animals should be conducted on a daily basis. Those subject to low welfare risk are to be minimally intrusive while those at higher risk require individual close-inspection using a graduated clinical scoring assessment system. Recommended monitoring requirements and endpoints for weight loss, clinical condition, and tumour size are presented.

As a default, unless weight loss is expected as a result of experimental protocol, an acute unexpected weight loss of 10%, within 48 hours, should require senior technician or Animal Welfare Officer (AWO) consultation. Weight loss of 15% and greater, detected at any time, should result in veterinary consultation and resulting advice followed. Weight loss anticipated to extend beyond 15% requires special justification in project applications with the harm-benefit costs addressed.

Absolute humane endpoints should include unrelieved respiratory distress, a body condition score (as defined by Ullman-Culleré and Foltz, 1999) of less than 2 out of 5, and ectopic subcutaneous tumours greater than 1688 mm^3 , for mice, based on recommendations from published literature (Workman et al, 2010). In addition, with justification and a strong harm-benefit cost analysis provided, humane endpoints may be set for tumours up to 2000 mm^3, provided their biology is understood and Animal Welfare Committee (AWC) approval is obtained. Endpoints for the size of orthotopic xenograft or spontaneously occurring tumours should be based on the localised tissue and systemic impact, which will vary depending on the tumour model and its biology.

Humane endpoints using clinical scores should be based on graduated objective clinical criteria with intervention points to respond to welfare impact at lower scores. Recommended criteria and objective descriptions of scoring are provided in the exemplar clinical coversheet with maintenance monitoring and individual clinical record sheets at the end of this document.

Expected adverse impacts on welfare are to be listed in AWC applications, and these impacts are tracked using Maintenance Monitoring Sheets, Clinical Record Sheets, and Running Mortality Sheets. Relevant data should be collated and presented in Annual or Final Reports. Use of this system will reduce the frequency of unexpected adverse events, and enable rational evidence-based decision making in welfare management.

2. Introduction

There is a need, in animal research, to maintain and develop consistent yet flexible welfare monitoring approaches with agreed uniform criteria, intervention, and humane endpoints. Researchers can then have confidence that the experimental design regimes and the animal care protocols they present to the AWC are of an acceptable standard. Adoption of these criteria will simplify and standardise monitoring regimes and reporting documentation, while reducing unnecessary animal handling and the work load of investigators and technical care staff.

Items addressed in these guidelines include:

- Clinical Record Sheet format with exemplar templates;
- Monitoring regimes at times of low versus high welfare risk;
- Common criteria for determining suffering in mice and rats in projects;
- Humane endpoints - including actions regarding weight loss, clinical condition and tumour sizes;
- The independence of the AWO in veterinary clinical decision making in the determination of intervention, humane endpoints, and emergency treatments; and
- Tracking and data collection of welfare impact in projects.

3. Clinical Record Sheet Format and Exemplar

Proposed Clinical Record Sheet documentation is presented at the end of this paper. These consist of a modified version of our existing Clinical Record Coversheet (derived from the University of Western Australia's clinical sheet cover sheet, see: https://www.research.uwa.edu.au/staff/forms/animals), an individual Clinical Record Sheet (presented with permission from the University of South Australia), and the existing Flinders Maintenance Monitoring Sheet.

4. Monitoring Regimes at Times of Low Versus High Welfare Risk

There is a welfare cost involved in the individual clinical monitoring of animals. Such a procedure involves taking the cage off an IVC rack, opening the lid in a cage change station, disrupting the nest to view animals, and may involve capturing and restraining individual animals in order to weigh or closely examine. There should be a recognition that some strains are extremely sensitive to interference, and that all mice and rats, since they are nocturnal, will have their sleep period disrupted when subjected to intensive individual monitoring in the day cycle. Such interference is not justified at times of low welfare risk.

4.1. Low Welfare Risk Monitoring

At times of low welfare risk whole-cage checks, as undertaken daily by animal facility staff, should be undertaken in which the cage environment and animals are viewed from outside the unit without disrupting the animals. Where there is suspicion that an anomaly is present, the cage should then be opened, the nest exposed, and individual animals checked. If any anomalies are detected in the animals or cage environment, these are either immediately corrected or the animals concerned are then transferred to individual Clinical Record Sheets, which involve daily monitoring. Intervention actions will result from clinical score tallies described in these sheets and monitoring will continue until the problem is corrected. This applies also to animals being transferred to researchers and prior to experimental interventions or before the welfare impacts of a disease model are likely to become

manifest. If experimental interventions or the disease model are mild and do not result in welfare impact then whole cage checks with sighting of individual animals not involving restraint should be undertaken in preference to individual animal monitoring and recorded on the Maintenance Monitoring Sheet (see Attachment 2). Such monitoring in low welfare risk situations should occur at a maximum frequency of once daily and, thus, researchers will need to liaise formally with animal facility management to determine whether the investigators or facility staff will undertake this activity.

4.2. High Welfare Risk Monitoring

Frequency of monitoring

The frequency of individual animal monitoring, incorporating palpation or imaging, for the purpose of the detection of such conditions as the presence or size of tumours should be based on welfare risk. There is the possibility that frequent monitoring and handling of animals with tumours may constitute confounding variables in tumour growth. Thus, frequent hands-on monitoring needs to be justified. For ectopic xenograft or spontaneous tumours in mice of subcutaneous regions with a low tendency to metastasise, as documented by experimental evidence (both published or unpublished and/or determined by suitable pilot studies), once weekly individual monitoring should occur until a tumour reaches 5 mm x 5 mm (65 mm^3)[1]* in size. The size specified at which more frequent monitoring is initiated may be larger or smaller depending on the biology of the tumour. Any deviation from the suggested 5 mm x 5 mm size for single tumours, or if multiple spontaneous tumours of less than 65 mm^3 contribute to a cumulative tumour burden that impacts on the clinical status of the animal these conditions, should be justified in the animal ethics application and approved by the AWC. More frequent individual monitoring devised in consultation with the AWO would then occur as the tumour progressively enlarges, or depending on the biology of the tumours, as determined by previous research or pilot studies. By contrast, with malignant tumours, evidence of respiratory distress such as dyspnoea is likely to represent a sign of pulmonary metastasis with a need to consider euthanasia to protect welfare.

Clinical monitoring

Individual clinical monitoring should occur after surgical interventions with general anaesthesia at the time of the regaining of righting reflexes, and then 2 hours later. Surgeries involving an extended time-period, such as greater than 20 minutes, or anaesthesia involving injectable agents should be accompanied by more vigorous monitoring involving frequent intermittent checking over the first 2 hours after the regaining of righting reflexes. An individual Clinical Record Sheet would then be completed two hours after the regaining of righting reflexes and again at the end of the day, prior to, or just after, the onset of the dark period. Monitoring should then occur twice daily on the next two days, prior to 10 o'clock in the morning and again at the end of the day, and then once daily thereafter if the welfare risk is expected to be low, during surgical recovery. In some studies involving reversal of the photo-period, or where the experimental protocols require interventions late in the day or during the night, a variation in the monitoring regime will be described in the animal ethics approval conditions. Well-established procedures, such as simple and limited cutaneous incisions,

[1] * Tumour volume = $4/3 \cdot \pi \cdot [r]^3 = 4/3. \pi .(2.5)^3 = 65.4$ mm^3 (M Samuel, Centre for Cancer Biology, UniSA, pers comm, 2019)

embryo transfer, or ovario-ectomies, are viewed as low risk procedures. Two occasions of monitoring on the day of surgery, as previously described, followed by daily monitoring on Clinical Record Sheets for 5 days are adequate for such low-risk procedures. The animals are then monitored once daily by whole cage checks documented with Maintenance Monitoring Sheets by the researchers, or if the researchers have delegated their responsibility to facility staff, cage checks can be performed by technical personnel.

Weight monitoring

The monitoring regime should also indicate the frequency of body weight measurement. For complex surgery, weighing should occur once in the immediate post-operative period, again at 48 hours, then daily for 3 days, and then once weekly. Capture and restraint within the first 48 hours of surgery for weight measurement purposes should be assessed on a cost benefit analysis by the consulting veterinarian, as it may contribute to additional stress for the animal. Weight measurement for routine surgery should occur in the immediate post-operative period, and repeated 5 days later. In well-characterised surgical models, weight loss of 10% or greater from the baseline should result in consultation with the veterinarian to determine if individual clinical monitoring until weight is gained is justified with respect to the cost benefit of close monitoring. In less-characterised surgical models, individual monitoring should continue when weight loss reaches 10% or greater.

Infectious-challenge or highly aggressive tumour models should have monitoring schedules determined by pilot studies with humane endpoints determined prior to the onset of the main study.

5. Common Criteria for Determining Suffering in Mice and Rats in Projects

Criteria to determine the presence of suffering from acute conditions such as surgery, first 24 to 48 hours, from Flecknel P (2015, p 168), include:

Mice: writhing, rear leg lift, belly-press, flinching, and facial grimace.

Rats: back arching, belly-press, fall/stagger, writhing, twitch, and facial grimace.

Criteria to determine presence of suffering from chronic conditions for rats and mice, beyond 48 hours, from Hankenson F C (2014, pp 30 – 31), include:

- Hunching and ruffled coat.
- Lack of spontaneous movement, and lack of response when gently touched.
- Skin crinkling, tenting, or sunken-eyed appearance.
- Social isolation or not nesting (mice).
- Pale extremities and/or cold to touch.
- Blood and/or mucopurulent (pus) discharge from body orifices.
- Laboured breathing.
- Profuse diarrhoea for 48 hours or more, or bloody/pus diarrhoea for any time span.
- Self-mutilation involving signs of excessive licking, biting, or scratching.
- Abnormal postures or behaviour – ataxia, circling, or dragging back legs.
- Large palpable unexpected masses.

5.1. Clinical Scoring to Estimate Welfare Impact and Guide Intervention

Clinical Record Sheets in animal research are used in an attempt to assess the presence of the above signs of suffering. Scores are allocated to the signs and then tallied to provide a number, against

which are matched, intervention actions involving additional monitoring, treatment, and veterinary consultation with further recommended actions. The University of Western Australia clinical record sheet format promotes graded assessment of any one clinical criterion so that any one criterion may contribute a larger impact to the total clinical score. This system has been implemented at Flinders University and appears to have been accepted by researchers and is now being implemented by UniSA. What is not consistent at Flinders University, however, is the requirement for the researcher to contact the AWO for a tally score of "2" when there may be two mild signs, each scored as "1", that contribute to the total number. Consistency with UniSA would occur if we were to request researchers to contact the AWO if any one sign was scored "2" or a total cumulative score of "3". An added action has been added for a total score of "5" that will involve immediate euthanasia, unless contrary to veterinary advice. This will assist in decision making when the institutional veterinarian needs to make a final decision regarding the fate of an animal.

6. Humane Endpoints - Actions Regarding Weight Loss and Tumour Sizes

6.1. Weight Loss

The laboratory animal literature does not appear to prescribe a strict humane endpoint weight loss percentage across studies. Rather, the recommendation appears to be targeted to food or fluid restriction studies. According to the NHMRC, *Guidelines to promote the wellbeing of animals used for scientific purposes*, 2008, part III, G6, "Animals that lose more than 10% bodyweight (acutely) or 15% bodyweight (longer term) on restricted food or fluid protocols should be removed from the research protocol. If an investigator requires more severe weight loss in an animal subject, special justification should be submitted to the AEC." In describing humane endpoints these guidelines also suggest that, "Animals that fail to adapt to the experimental conditions…(and those) … showing signs of dehydration … reduced food consumption … are to be removed from the research protocol."

Another important point to consider are the requirements of the *Australian Code* (2013) with respect to endpoints. Section 3.1.26 states that if pain and distress are predicted or unavoidable consequences of a project, validated criteria must be established to identify when intervention is necessary to minimise pain and distress.

Mellor et al (2009) states that the capacity to assess welfare status and to grade the nature and degree of compromise caused by such investigations are central to maintaining societal support for scientific projects in which the anticipated benefits substantially outweigh the negative impact on animals when all practicable measures have been taken to minimize that harm. In this publication the authors grade food intake restriction causing weight loss up to 20% as being of a "C" grade consisting of a moderate negative impact in two of the five domains of animal welfare, including the mental domain. This "C" grade is applicable to studies in which unpleasant experiences occur at moderate levels for short periods, or at low levels for long periods. In disease models, where weight loss is greater than 20%, the level of welfare compromise begins to stray into Mellor's "D" category. This category is applicable to studies in which unpleasant or noxious experience are marked and where the suffering that is caused may end in euthanasia or by therapeutic interventions before it becomes excessive.

Higher weight loss scores may be acceptable when previous experience has shown that, in a given experimental protocol, this weight loss is likely to be rapidly reversed, such as over a 5 to 7 day period.

Weight loss in such studies may be due to the effect of the loss of body water through the disease process or because of a failure to eat or drink due to the experience of malaise. An important factor in rodent weight loss is that contributed by "dehydration anorexia" as described by Rowland (2007). In the early period of water deprivation, rodents will significantly reduce their food intake. Such early weight loss is associated with the emptying of the gastro-intestinal tract and the subsequent depletion of body store depots in the form of fat and muscle. Significant weight loss attributed to dehydration occurs in the terminal stages of deprivation. Therefore, the loss of body depot stores (muscle and fat) and body water can be used to monitor the progression of welfare compromise if animals do not drink or eat sufficiently to maintain bodyweight.

Bodyweight loss, in itself, can be a blunt and an inaccurate measure of clinical and welfare status. This opinion is supported by observations expressed in published literature. Ullman-Cullere and Foltz (1999) allude to the practical problems associated in the measurement of bodyweight in rodents and indicate that it may not yield an accurate measure of fat stores and muscle mass. Rather, Ullman-Cullere et al (1999) promote and provide a tool for measuring body condition in mice. This scoring system has been promoted by contemporary leading textbooks relating to laboratory mouse husbandry. These publications include those of Wolfensohn and Lloyd (2013, p 46), and Hankenson (2014, pp 29 – 30).

6.2. Recommendations Regarding Weight Loss

The recommendation to the AWC from the AWO is that the decision to allow projects to be authorised where weight loss is greater than 15% is an ethical decision in which the benefits of the research is balanced against the harm caused to the animals concerned. The risk of animal welfare compromise increases as bodyweight loss becomes progressively more severe.

For projects where weight loss is not expected, the NHMRC Guidelines may be used as a guide, such that if an animal loses 10% of its bodyweight a senior technician should be consulted to confirm the clinical assessment of the animal. Note that weight measurement, in a study in which weight loss is not expected, is unlikely to occur daily, however, an animal that loses 10% of its weight acutely, is likely to be suffering from dehydration and will demonstrate other clinical signs. The AWO should then be notified and veterinary advice followed if any clinical compromise is noted. If weight loss of 15% or over, as determined from a reference weight, is noted at any time, then the AWO needs to be consulted and veterinary advice followed. If the AWO cannot be contacted, then the animal should be euthanised.

Note that the suggested intervention points set at 10% for acute and 15% chronic weight loss are more rigorous than those described in the literature. Hankenson F (2014, p 175) recommends veterinary intervention at 20% in mice and rats and Workman et al (2010, p 1571) refers to 15 to 20% in tumour studies. McGill University Canada in its SOP #415 *Humane intervention points for rodent cancer model* lists a clinical intervention point of 20% weight loss in adults, while Rowland (2007, p 159) states that food deprivation studies leading up to 15% weight loss is acceptable.

6.3. Tumour Size

Tumours may contribute to animal suffering through their physical expansion and effect on surrounding tissues through direct pressure, disruption of blood supply, infection, and inflammation, or by impeding an animal's behavioural activity. Other effects on the body may become significant when the tumour spreads from its primary origin, in the form of metastases, or as a spontaneous[1] or transplanted tumour where their locations disrupt body systems. This is especially the case in spontaneous[1] or orthotopic[2] tumours. The severity of welfare impact depends on the tumour biology

and the location of the tumour in the animal. Cachexia, a condition often associated with malnutrition and poor body condition, may be seen with advanced orthotopic tumours. Such problems require careful monitoring through body condition scoring, since, "… an animal's bodyweight, as on its own it may not be a reliable indicator" (NHMRC Guidelines, pp M3). Other side effects of tumours that result in malaise, lethargy, and pain are measured using standard clinical criteria common to other research projects. These include hunching/ruffling, skin crinkling/tenting, sunken eyes, lack of movement, social isolation, and pale/cold extremities.

It should be noted that "Rodents can sustain, large, superficial, non-invasive tumours without any apparent effects or restriction on their normal behaviour" Wallace (2000, p 89). Examples of such tumours include xenograft[3] ectopic[4] tumours that are implanted under the skin.[2]

Projects conducted under the aegis of the UK Institute of Cancer Research comply with the guidelines enunciated by Workman et al (2010) for superficial tumours, which are, "For an animal carrying a single tumour, the mean diameter should not normally exceed 1.2 cm in mice or 2.5 cm in rats, or 1.5 and 2.8 cm, respectively, for therapeutic studies". These dimensions, regarding therapeutic studies in mice, result in a tumour volume endpoint of 1688 mm^3 using criteria promoted by Faustino-Rocha et al (2013).

It is significant that Workman et al (2010) make no reference to evidence, nor other publications, in making the recommendation. Not all institutions follow such a cut off. In similar jurisdictions to Australian, McGill University in Canada uses a humane endpoint of 2000 mm^3 as described in in its SOP #415 *Humane intervention points for rodent cancer model*.

6.4. Tumour Size Recommendations

It is recommended that as a general guideline, for ectopic and spontaneous tumours in mice, the conservative figure of 1688 mm^3 be used for therapeutic studies. In addition with justification, tumours may be approved up to 2000 mm^3, provided their biology is understood based on published evidence or previous pilot studies, and their placement position on the body does not impede locomotion. For ectopic and spontaneous tumours measuring over 1000 mm^3, an examination should be undertaken weekly by an independent qualified senior technician in consultation with a veterinarian, or directly by a veterinarian, to confirm that the welfare of the affected animals is within the parameters set by the AWC.

Large discrete subcutaneous tumours often grow beyond the capacity of their blood supply to sustain them, with resulting ischaemic-hypoxic tumour cell death, particularly in core regions. This feature of cell death within the tumour may also occur due to experimental tumour treatments. The death of the central tissue within the tumour may result in the collapse of the surface of the tumour, which then appears as ulceration and cavitation. This ulceration and cavitation in itself may not represent a welfare impact. It is suggested that the tumour ulcer criteria derived from the QIMR Berghofer Medical Research Institute for subcutaneous ectopic tumours be used as a guide for the determination of humane endpoints. Criteria of humane endpoint involves a wet tumour surface or

[1] Spontaneous – A spontaneous tumours are those which arise in an animal without intervention. Thus, these tumours arise without any control of the site location and there growth and biology may be difficult to predict.
[2] Orthotopic – Anatomically correct site, such as renal tumour cells implanted into the kidney
[3] Xenograft – Foreign tumours cells transplanted between species
[4] Ectopic – The site of tumour growth is different from the tissue of origin, such as tumour cells from internal body organs transplanted by subcutaneous injection.

bleeding evident which does not dry or scab within 6 hours or overnight or there are accompanying signs of ulcer inflammation or persistent scratching or cleaning by the mouse.

In tumour studies, any assessment of body weight loss, especially tumour-induced cachexia, will need to take into account any increased body weight due to an enlargement of the tumour mass. For instance, it is assumed that a tumour of 1000 mm^3 is of the same density as other body tissue and would weigh one gram.

7. The Independence of Veterinary Clinical Decision-Making

Veterinarians by means of their training and registration with Veterinary Surgeons Boards are professionals qualified and deemed expert in the diagnosis and treatment of animals. Veterinary practitioners are responsible for their own actions and judgements. Allowing their professional judgement, integrity, discretion, conduct, or ethical standards to be compromised by any other person in any matter requiring the application of professional knowledge or skill is not a defence against allegations of unprofessional conduct (Veterinary Practitioners Registration Board of Victoria, 2015, p 38, guideline 19.1).

Animal Ethics Committees allow veterinarians, if consulted by researchers or technicians, to exercise discretion in the interpretation of clinical signs and intervention points in research projects. This particularly is the case with humane endpoints relating to weight loss and tumours if unaccompanied by clinical signs or, where clinical signs are ambiguous.

In addition, veterinarians attending the animal facility may need to make decisions in an emergency to protect animal welfare, maintain the scientific integrity of projects, or act to implement disease prevention and control measures.

The University employs an AWO, who is required to be a registered veterinarian with expertise in laboratory animal medicine. The AWO provides specialist expertise in the management of animal welfare and veterinary treatment related to research and teaching conducted by University staff and students.

In such emergencies, the AWO may need to act without prior AWC approval. If this occurs, the AWO should inform the Chair as soon as possible, and inform the AWC at the next full AWC meeting via a detailed AWO report.

8. Tracking and Data Collection Strategy in Projects

One of the purposes of an Animal Ethics application is for the research applicant to declare the harm that may result from experimental intervention and then balance that harm by explaining the benefits of the research (a cost/benefit analysis). Researchers should clearly describe in their applications the expected deleterious animal welfare impacts of the experimental interventions. This avoids incidents involving these impacts being classified as unexpected adverse events. An "unexpected adverse event" is defined as one that was not anticipated, and flagged in the current application, when the experimental protocol was compiled. Such undesirable effects of research may result in animals either receiving high clinical scores when monitored, or reaching humane endpoints that prompt humane killing or dying when their condition deteriorates rapidly between monitoring checks.

Other causes of death and euthanasia may be due to anaesthetic and surgical complications, or to attrition rates in short-lived animals which may be exacerbated by deleterious phenotypes associated with inbreeding or gene modification. It is essential that data describing the number of animals reaching humane endpoints or mortalities are collected so that facility management, researchers, and the AWC can apply more accurate predictions of welfare impact and loss of research animals in projects. Researchers should be encouraged to nominate a “loss rate” or “mortality rate” based on in-house data, publications, or well respected opinion. In those projects involving high welfare impact, they should subsequently track those losses by maintaining running-mortality tally sheets (see Attachment 3). Such data should be included in Annual and Final Reports submitted to the AWC.

9. Attachment 1: Clinical Record Coversheet & Clinical Record Sheets *(current as of 09/06/20)*

Flinders University
Exemplar Individual Clinical Record Coversheet

(Clinical Record Coversheet adapted with the permission of The University of Western Australia, Office of Research Enterprise. ["Monitoring Cover Sheet" at http://www.research.uwa.edu.au/staff/forms/animals])

Project Number	
Project Title	
Chief Investigator	
Monitoring Start Date / Animal Issue Date	

1) CONTACT DETAILS

Contact Type	Name	Contact Number
Emergency Contact		
Researcher (1)		
Researcher (2)		
Researcher (3)		
Animal Facility Staff		
Animal Welfare Officer	Lewis Vaughan	0450 424 143
Other (please specify)		

2) SPECIES / PHENOTYPE / MODEL ISSUES

EXAMPLE: In NSG mice some ruffling of coat is normal
EXAMPLE: Diarrhoea is expected during chemotherapy and is scored as normal if not profuse and blood or mucus is absent.

3) MONITORING CRITERIA

CRS Column #	Clinical Criteria	No obvious deviation from normal	Slight or moderate deviation from normal	Significant or sustained deviation from normal
	Score	**0**	**1**	**2**
1	Lack of, or abnormal movement	Active spontaneous free movement	Lack of spontaneous movement/hyperactivity/occasional ataxia /circling/dragging legs	Reduced movement on stimulation/frequent ataxia, circling, locomotion defects
2	Posture	Extended back, normal movement and gait	Slight arching on movement	Arched on movement
3	Ruffled coat	Smooth sleek coat	Slight ruffling	Significant ruffling over entire coat
4	Abnormal breathing	Normal breathing in character and frequency	Increased respiratory rate or slight increased effort	Marked increase in respiratory rate with marked effort
5	Skin crinkling/tenting/sunken eyes	Soft, pliable, elastic, bright eyes (> 75% open)	Skin crinkled, slight tenting when skin lifted	Skin tenting and sunken eyes

6	Injury	No incision anomaly or evidence of injury	- (Not scored – all significant injuries are to be reported to the AWO)	Significant injury/body cavity penetration/ skin excoriation ≥ 1 cm^2/scratching/obvious swelling/ discharge/gaping wound
Optional additional criteria are shown below – These should be included if applicable to the project, or interchanged with project specific criteria. Example: tumour-scoring criteria for tumour studies.				
7	Abnormal discharge from orifices	No discharges	Slight discharge (blood/pus etc) from any orifice	Significant discharge (blood/pus etc) from any orifice -
8	Profuse/bloody diarrhoea	Well-formed stools	Profuse with cage smeared with diarrhoea	Bloody & profuse diarrhoea
Note: Training by the AWO or pictures or video footage of the monitoring criteria at the various scoring points is recommended to ensure all personnel are consistent in terms of scoring.				

4) MONITORING AND WEIGHING FREQUENCY

Describe monitoring regime.
All animals receiving procedures will be clinically assessed, using individual CRSs, 2 hours after anaesthetic recovery, at the end of the day, then twice daily for 2 days, then once daily for 5 days. If no clinical scores are observed the animal will be transferred to daily maintenance monitoring. Animals are to be weighed on the third day after surgery and then once weekly.

5) ACTIONS AND INTERVENTIONS

Total Score	Animal Assessment	Actions/Interventions
0	Within normal limits	No interventions required.
1	Slight or moderate deviation from normal	Provide analgesia if indicated, consult AWO if in doubt. Daily monitoring required
2	Moderate abnormality	Score 2 for any one criterion – consult AWO Cumulated score 2 – increase monitoring to twice daily
3	Moderate/sustained abnormality in more than one criterion	Consult AWO, follow advice Increase monitoring to three times daily
4	Significant or sustained abnormality	Immediately euthanise or consult AWO and follow advice ▪ Complete a Running Mortality Sheet. ▪ If welfare compromise or mortality rates fall outside of approved conditions (section 3.7 of the application), an Unexpected Adverse Event (UAE) Report must be submitted.
5	Severe abnormality	Immediately euthanise the animal, unless contrary to veterinary advice.. • Mortality and UAE reporting as for "4" above
Absolute humane endpoint criteria		
Laboured breathing	Animal exhibits respiratory distress – gasping, blue membranes	

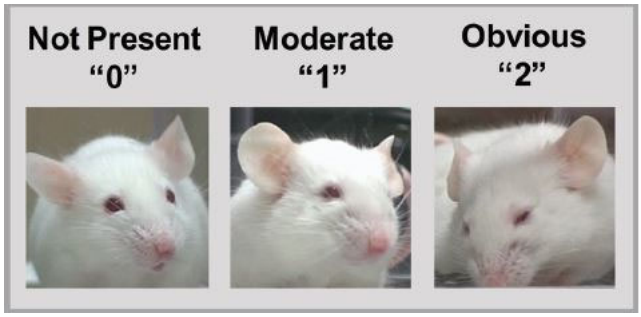
Body condition score (BCS) < 2/5	BSC < 2/5 signifies sharp vertebral processes, body is emaciated	
Weight loss %	Weight loss exceeds 10%, but is less the 15%	Correct sipper water flow, give soaked food Increase monitoring frequency to twice daily. Consult senior facility technician, notify the AWO depending on technician advice. If 10% weight loss occurs within 24 hours then consult AWO and follow advice .
	Weight loss exceeds 15% or 20% (depending on ethical approval)	Adhere to conditions of ethical approval or consult AWO and follow advice or immediately humanely kill. .
Other recommended project specific, criteria and actions		
Mouse/Rat grimace score **(See illustration at end of document)**	Mouse grimace "moderate" or "obvious" as per Matsumiya et al 2102, JAALAS	If within 48 hours of acute trauma administer pain relief. If beyond 48 hours consult AWO and follow advice.
Tumour size	Ectopic subcutaneous tumour – one tumour or cumulative tumour burden of 1000 mm^3 or greater	Consult AWO and follow advice. AWO to monitor as per ethical approval.
Tumour size	Ectopic subcutaneous tumours – one tumour or cumulative tumour burden, greater than 1688 mm^3 as per Workman et al, 2010	Follow conditions of ethical approval or consult and follow AWO advice.
Tumour ulceration	Wet tumour surface or bleeding evident which does not dry or scab within 6 hours or overnight/ accompanying signs of ulcer inflammation/ persistent scratching or cleaning by the mouse	Adhere to conditions of ethical approval or consult AWO and follow advice or immediate euthanasia. Complete a Running Mortality Sheet.
Tumour position	Position of the tumour significantly impedes physiological function or locomotion	Consult AWO and follow advice. AWO to monitor as per ethical approval.
Note: Weight loss in growing animals is calculated against pre-interventional weight, control animals or growth-charts. If growing animals fall below the bottom of the growth curve for the background strain as provided from the supplier, consult the AWO or humanely kill.		

6) INSTRUCTIONS

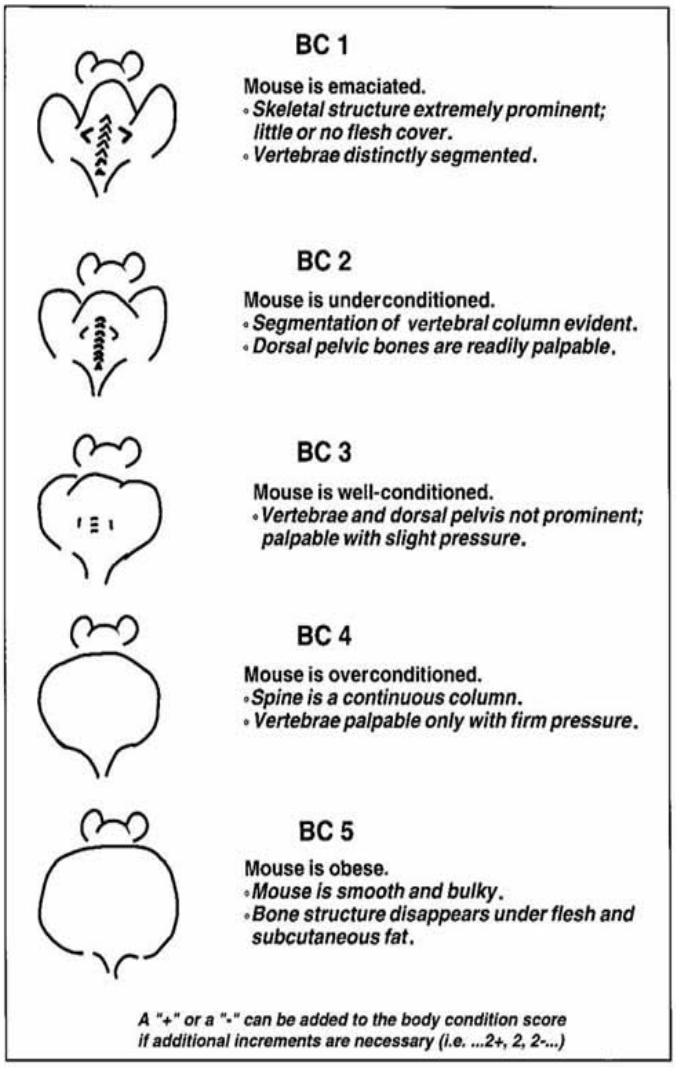
a. Each animal is examined at each nominated monitoring time point.
b. Each criteria is scored and the score marked on the record sheet. Training by the AWO is required to ensure all personnel are consistent in terms of scoring.
c. Scores are then added together and a total score is recorded on the record sheet.
d. Specific actions/interventions are undertaken in accordance with the clinical score.
e. Comments concerning abnormalities are recorded in the "Comments" section.

f. Any other abnormalities are recorded in the "Other" section.
g. Any abnormality that is of greater severity than the descriptions above, or is a major deviation from normal, beyond that approved, will require immediate consultation with the AWO or immediate euthanasia with submission of an Unexpected Adverse Event report.
h. All unexpected adverse events must be reported immediately to the AWO and an Unexpected Adverse Event Report submitted to the Animal Ethics Officer.

Mouse grimace score



From Matsumiya et al, 2012, Using the Mouse Grimace Scale to Re-evaluate the Efficacy of Postoperative Analgesics in Laboratory Mice, *JAALAS*, Vol 51, No 1, January 2012, pp 42 - 49



From Ullman-Culleré M H and Foltz C J, 1999, Laboratory Animal Science, Vol 49, No 3, June 1999

Flinders University (Acknowledgement: University of South Australia)

EXEMPLAR SINGLE ANIMAL CLINICAL RECORD SHEET *– See coversheet for detailed descriptions of clinical criteria, actions, monitoring and weight frequencies.*

Study Name:

CHIEF INVESTIGATOR:		WORK PHONE:		AFTER HOURS PHONE:	
RESEARCHER:		WORK PHONE:		AFTER HOURS PHONE:	
ETHICS NO:		CAGE NO/ANIMAL ID:		AWO NAME/CONTACT	L Vaughan 0404721720
COMMENCEMENT WEIGHT (g)		90% OF COMMENCEMENT WEIGHT:		85%/80% (STRIKE OUT WEIGHT LIMIT NOT APPLICABLE) OF COMMENCEMENT WEIGHT:	

		1	2	3	4	5	6	7 OPTIONAL	8 OPTIONAL				(OPTIONAL)		
DATE	**TIME**	**MOVEMENT**	**POSTURE** (Hunching)	**RUFFLED COAT**	**ABNORMAL BREATHING**	**SKIN TENTING/ SUNKEN EYES**	INJURY	DIARRHOEA – BLOODY/PROFUSE	**DISCHARGES**	TOTAL SCORE	COMMENTS – OTHER CLINICAL SIGNS AND ACTIONS	BCS X = Less than 2/5	MOUSE/RAT GRIMACE SCORE (M/RGS) (Within 48 hours of surgery) X = present	WEIGHT (G)	SIGN

See Coversheet for detailed descriptions of clinical criteria and actions and monitoring/weighing frequencies

Actions for scores:

1 – Monitor daily; **cumulative 2** – Monitor twice daily, **2 for any one criteria** – consult AWO, follow advice; **3** – Consult AWO monitor 3 times daily; **4 or over** – Immediate HK or consult AWO.

M/RGS: X (present) – provide pain relief as specified in ethical approval or as per AWO advice

Weight loss: 10% or greater -check sipper tube, give soaked food, consult senior technician and monitor twice daily where applicable

Weight loss: 10% within 24 hours or over 15/20% (strike out non-applicable weight loss) over 24hrs – consult AWO or HK, depending on ethical approval.

ABSOLUTE HUMANE ENDPOINTS: laboured breathing; BCS < 2/5. **IF IN DOUBT OR CONCERNS CONTACT AWO**

Flinders University (Acknowledgement: University of South Australia)

EXEMPLAR CAGE-BASED CLINICAL RECORD SHEET

ETHICS NO		**COMMENCEMENT DATE**		**CAGE ID**	
EXPIRY DATE				**STRAIN/SEX/DOB**	
RESEARCHER		**PHONE**			
AWO		**PHONE**			

See coversheet for detailed descriptions of clinical criteria, actions and monitoring/weighing frequencies

Guinea pig ID	Commencement weight (g)	Weight at 10% loss (g)	Weight at 15/20% loss (g)

DATE (20__) TIME	ANIMAL ID	WEIGHT (g)	CLINICAL SIGNS NORMAL = 0 (blank); ABNORMAL MODERATE = 1, SIGNIFICANT = 2								TOTAL SCORE (TOTAL		COMMENTS/OTHER CLINICAL SIGNS AND ACTIONS (Record if AWO or senior COMPHAF technician consulted)	INITIAL
			1	2	3	4	5	6	7	8				
			MOVEMENT	POSTURE	RUFFLED COAT	ABNORMAL BREATHING	SKIN TENTING /EYES SUNKEN	Injury	DIARRHOEA BLOODY/PROFUSE	DICHARGES		BCS (X = less than 2/5)		
AM/PM														
AM/PM														
AM/PM														
AM/PM														
AM/PM														

Actions for scores:

1 – Monitor daily; **cumulative 2** – Monitor twice daily, **2 for any one criteria** – consult AWO, follow advice; **3** – Consult AWO monitor 3 times daily; **4 or over** – Immediate humanely kill (HK) or consult AWO.

Weight loss: 10% or greater -check water supply, give soaked food, consult technician and monitor twice daily where applicable

Weight loss: 10% within 24 hours or over 15/20% over 24 hours (strike out non-applicable weight loss) – consult AWO or HK.

ABSOLUTE HUMANE ENDPOINTS: laboured breathing; BCS < 2/5. IF IN DOUBT OR CONCERNS CONTACT AWO

DATE (20__) TIME	ANIMAL ID	WEIGHT (g)	CLINICAL SIGNS NORMAL = 0 (blank); ABNORMAL MODERATE = 1, SIGNIFICANT = 2								TOTAL SCORE (TOTAL		COMMENTS/OTHER CLINICAL SIGNS AND ACTIONS (Record if AWO or senior COMPHAF technician consulted)	INITIAL
			1	2	3	4	5	6	7	8				
			MOVEMENT	POSTURE	RUFFLED COAT	ABNORMAL BREATHING	SKIN TENTING /EYES SUNKEN	Injury	DIARRHOEA BLOODY/PROFUSE	DICHARGES		BCS (X = less than 2/5)		
AM/PM														
AM/PM														
AM/PM														
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AM/PM														
AM/PM														
AM/PM														

Clinical record sheet adapted and reproduced with permission from the University of South Australia, Research and Enterprise. (Monitoring Exemplars CRS Cage Individual, see: https://mymailunisaedu.sharepoint.com/teams/rch/ris/animalethics/Pages/aec.aspx

10. Attachment 2: Maintenance Monitoring Sheet *(current as of 09/06/20)*

Flinders University
Maintenance Monitoring Sheet

1) ANIMAL DETAILS

AEC Project #		Monitoring frequency	Daily
Name of CI and researchers		Strain	
Room number		Number of Cages in Room	

2) MONITORING

Date										
Time										
Number of cages										
Criteria										
Behavioural abnormality										
Physical abnormality										
Cage environment abnormality										
Total										
Signature										
OFFICE USE ONLY **AWO CHECK**										

Scoring criteria:

- Yes = 1 - Behavioural abnormality – no activity, abnormal activity, poor nest formation, abnormal gait, hunching, facial grimace
  - Action required – commence twice daily monitoring with post-procedure clinical record sheet
- No = 0 – Behavioural normal – bright and alert, active, resting/sleeping in nest, normal gait, absence of hunching/facial grimace
- Yes = 1 – Physical abnormality – presence of lesions, asymmetry, swellings, low body condition score (less than 2 out of 5- see next page for description)
- No = 0 – Physically normal
- Yes = 1 – Cage/environment abnormality – air flow, temperature, humidity, contamination, moisture, ammonia
- No = 0 – Cage/environment normal - air flow, temperature, humidity, contamination, moisture, ammonia

If a score of 1 is recorded for any of the criteria, monitoring with post-procedure Clinical Record Sheet will commence.

Cages identified for twice daily monitoring (indicate method of cage identification)	

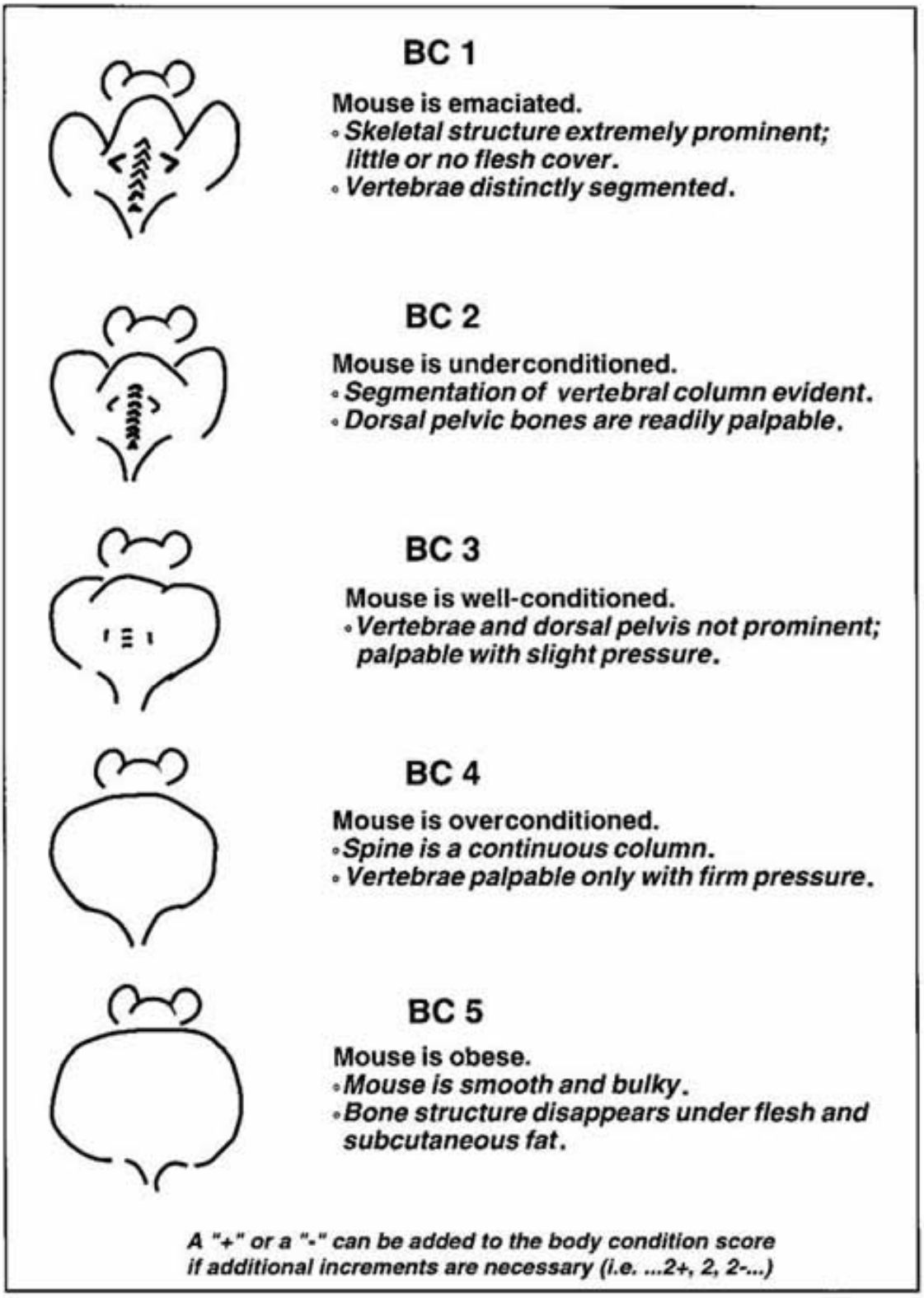

Fig. 3.2 Schematic for scoring of the mouse body condition (from Hankenson CF 2014, *Critical Care Management for Laboratory Mice and Rats*, CRC Press, Florida.)

11. Attachment 3: Running Mortality Sheet *(current as of 09/06/20)*

Flinders University Animal Welfare Committee

Running Mortality Sheet – for mortalities specified in approved projects
College of Medicine and Public Health Animal Facility

Project Number: **Chief Investigator:**

Project Component (if applicable):

Reporting Requirements: The AWO must be contacted promptly following _____ or more consecutive mortalities. Once the mortality rate exceeds the approved predicted rate, an Unexpected Adverse Event Report must be submitted to the Animal Ethics Officer for each subsequent mortality. The Running Mortality Sheet must be submitted as part of the yearly Annual & Final Reports for each project. Please consult with the AWO if a death or euthanasia is associated with conditions outside of what is specified in the ethical conditions of your project. This may be an Unexpected Adverse Event (UAE), and thus the AWO may request you to submit an UAE report.

Total Number of Animals in Project/Component =_____ animals
Approved Mortality Percentage =_____ % =_____ animals

Date	Time	Death Classification *(See definitions on following page)*	Housing (individual, group, indoor, outdoor) / Wild Habitat	Nº Euthanised Reaching Humane Endpoint	TALLY Nº Euthanised Reaching Humane Endpoint to Date	Nº Found Dead	TALLY Nº Found Dead to Date	Total Nº Animals Used in this Project to Date	Total Mortalities to Date	Mortality % *(calculated against total numbers approved)*	Signature

Death classification	Definition of death attribution
Anaesthesia	Anaesthetic complications
Cage environment	Cage/enclosure conditions
Cannabilism	Conspecific with consumption of part of or complete carcass
Conspecific aggression	Aggression from cage mates or wild conspecifics
Euthanasia	Humane endpoint implemented to alleviate suffering
Experimental intervention	Expected adverse consequent of experimental intervention described in the AWC application
Handling – capture	Capture process
Handling – restraint	Animal death attributed to handling post capture
Illthrift	Poor body condition – death associated with reduced body fat and muscle stores
Infectious disease	Pathogen infection/infestation
Killed humanely	Animal killed as a result of colony management, collection of samples, end of study
Misadventure	Unexplained trauma
Predation	Killed by predator
Senescence	Ageing
Treatment	Therapeutic or surgical intervention for treatment purposes
Tumour	Complications associated with tumours
Unknown	Animal found dead with no known association
Weather	Weather conditions

Please contact the AWO or Animal Ethics Officer if you require clarity of wording or how best to adapt this form to your project.

12. References

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