ANIMAL CARE AND USE STANDARD

The Animal Care & Use Standards are designed to provide guidance regarding good practice to institutional animal users and carers, as well as Animal Ethics Committees (AECs), on the care and use of animals for scientific purposes such as research and teaching. The Standards are evidence-based, reflecting current or accepted good practice and allow for the flexibility that is required in research and teaching activities using animals.

MONITORING MICE POST IRRADIATION

This standard has been developed by the University of Melbourne Animal Care & Use Standards Committee, and endorsed by the University of Melbourne Animal Welfare & Ethics Committee.

V1 Date of Approval: 4 April 2016  
Date of Review: 4 April 2016

1. ASSOCIATED STANDARDS

This standard should be read in conjunction with the following University of Melbourne Animal Care & Use Standards:
- Humane killing of mice and rats

2. SUMMARY

2.1 Gamma irradiation is a type of ionizing radiation used to eliminate mitotically active cells by breaking the DNA helix. Rapidly dividing cell lines such as haematopoietic tissue and those lining the gastrointestinal tract (GIT) are most commonly affected.

2.2 Whole-body irradiation treatment is one of the most common tools for myeloablation (destruction) of the recipient's bone marrow.

2.3 Myeloablation results in severe suppression of the immune system. Immunological constitution requires an appropriate number of non-irradiated donor mice to provide sufficient haemopoietic cells/tissue for transfer to the irradiated donor. Reconstitution of irradiated mice is required unless specific justification is provided in the application and approved by the Animal Ethics Committee (AEC).

2.4 Dosages of irradiation can be lethal or sublethal. General monitoring will be the same for both dosages.

2.5 Monitoring regimes specifically designed for irradiated animals are critical in identifying welfare issues that can develop from the use of this treatment

3. BENEFITS & RISKS

3.1 Major benefits derived from irradiation based studies are the ability to study the complex components of the immune system and their interactions, using the murine host as a model for the human immune system.

3.2 The irradiation treatment is non-invasive, but not innocuous, and in most instances can be performed without removing the mouse from its own cage environment to minimise the stress associated with transport.

3.3 Animals are not observed to display any signs of acute pain associated with the procedure though general malaise in the post-irradiation period is common.

3.4 Whole body irradiation is not selective in the cells it targets. In addition to bone marrow damage, the GIT epithelium may be damaged leading to increased susceptibility to opportunistic infections and bacterial translocation from endemic gut microbes.

3.5 Risks and complications following gamma irradiation in mice include: weight loss, anaemia, infection,
gastrointestinal haemorrhage, transplant failure, graft versus host disease (GVHD), secondary neoplasia (long term studies only) and incisor damage.

3.6 A major aim of monitoring animals post irradiation is the early detection of failure to successfully engraft and pre-emptive euthanasia before the animals become moribund.

3.7 Although successful bone marrow reconstitution should be protective after 21-28 days, full immunocompetence may not be achieved until 6-8 weeks post-transplant. It is expected the transfer will occasionally be unsuccessful due to technical error or other causes. Where engraftment has failed, animals require euthanasia, especially if a lethal dose of irradiation was given.

4. PROCEDURE/PROTOCOL

4.1 Post irradiation antibiotic support

4.1.1 Antibiotic therapy is instituted to protect the recipient animals against bacterial translocation and opportunistic infection post-irradiation. The risk is greatest after day 7, so studies of less duration may not require their use.

4.1.2 It may take up to 28 days following irradiation and haemopoietic cell transplant to regain immunological competence in the recipient animal. A treatment period of 28 days is suggested, with an additional 1-2 days of use prior to the irradiation procedure to allow the mice to become familiar with the taste.

4.1.3 A broad spectrum antibiotic with effective coverage for gastrointestinal microbes such as *Pseudomonas aeruginosa*, *E. coli*, *Clostridium sp.*, and *Klebsiella* and easily administered in drinking water is recommended. Individual oral gavage may be used to administer antibiotics though is not recommended due to the increased stress for the animal and more intensive labour requirements.

4.1.4 Antibiotic drug options include Metronidazole, Trimethoprim/Sulfonamide, Neomycin, or Ampicillin as these have a good spectrum of activity against the microbes of concern. Specific dosing protocols can be obtained via discussion with the Animal Welfare Officer (AWO).

4.1.5 Acidification of water using hyper-chlorination to obtain a pH of 2.5-3.0 may be utilised to reduce bacterial contamination of drinking water sources offered to mice post-irradiation. Treatment of water with UV light, by autoclave or filtering by reverse osmosis (RO) may also be considered for this purpose.

4.2 Post irradiation reconstitution

4.2.1 In a successful host graft, hematopoietic repopulation is expected to occur within 14-21 days and should be protective after 21-28 days. Strict aseptic housing and husbandry procedures should be maintained for this duration.

4.2.2 All rodents do not respond identically when exposed to irradiation; many biologic factors potentially can affect the murine response to radiation.

4.2.3 Classic signs of discomfort and ill-thrift need to be monitored in all mice. These include, but are not limited to: ruffled fur, hunched posture, lethargy, squinting of the eyes, reduced mobility, difficulty breathing, diarrhoea or ongoing loss of body weight and body condition.

4.2.4 Animals should not be allowed to reach a moribund state; intervention points should be triggered before this to prevent suffering. If this occurs then reassessment of the monitoring criteria and frequency must occur.

4.3 Post irradiation monitoring

4.3.1 Record keeping charts, copies of the observation and intervention criteria and the body condition scoring chart should be readily accessible and held in the same room as the mice for ease of use. An example of a monitoring chart for use post- irradiation is provided in Appendix II. Further general templates are available via provided on the website of the Office for Research Ethics and Integrity: http://orei.unimelb.edu.au/content/forms/templates-guidance-documents
4.3.2 Actions to be implemented by animal house technicians are listed where appropriate on the relevant intervention sheet, but if in any doubt the Animal Facility Manager (AFM)/AWO should always be consulted.

4.3.3 Where a researcher is considering using monitoring criteria that they have not previously used, they should seek clarification on scoring from the AFM/AWO.

4.3.4 A base weight should be established for each individual rodent on day 0, prior to any planned irradiation and/or graft procedure.

4.3.5 The Body Weight (BW) in conjunction with the Body Condition Score (BC) of irradiated rodents is utilised as a method of monitoring their response and recovery following the procedures. Use of body weight alone is insufficient as a monitoring tool, as true loss of fat and muscle tissue may be masked by other factors, such as lymphoid tissue growth or fluid.

4.3.6 Rodents that undergo irradiation typically lose a considerable amount of weight, only to gain it back relatively quickly after successful transplantation. Therefore, animals may be permitted a weight loss of around 15%, but no more than 20%.

4.3.7 In general, more than 90% of reconstituted rodents should recover their weight loss by 21 days post-irradiation. Animals who do not meet this criterion should be considered at risk of transplant failure.

4.3.8 Animals should be weighed a minimum of twice weekly over the first 21 days post-irradiation. More frequent visual monitoring and weighing may be needed in some investigations.

4.3.9 Radiation sickness may be expected to affect irradiated mice from day 5-14 post treatment. The degree of severity of radiation related illness may vary considerably among individuals, and the monitoring criteria and intervention criteria are designed to identify mice that may progress to the irreversible end of the spectrum (i.e. failed bone marrow transplant) before it occurs.

4.3.10 The majority of animals will experience weight reduction and clinical illness corresponding to this time, so easy access to water through use of long sipper tubes or gel packs and additional soft food on the cage floor should be the standard of care during this period.

4.3.11 An increased frequency of visual monitoring and weighing is required for all animals once weight loss reaches 10% of starting weight, as outlined in section 4.4.2. This must continue until weight returns to 90% of the baseline. The AMF/AWO must be consulted immediately if weight loss exceeding 15% occurs after day 7.

4.3.12 Failure to gain or maintain weight after day 28 suggests a very poor prognosis and humane euthanasia should be considered for these mice

5. MONITORING & INTERVENTION

5.1 Irradiation and reconstitution procedures

5.1.1 Mice are generally left in their cage for transport to the radiation facility to minimise stress. Where possible, it is desirable but not essential that the mice are housed at the research facility for a few days prior to beginning any experimentation to allow them to acclimatize to the environmental conditions.

5.1.2 Immediately post-irradiation mice should be visually checked through the cage, but no direct adverse reactions are anticipated at this point. Mice should be moving about normally and show normal breathing patterns and effort.

5.1.3 Allow the mice to settle after irradiation and any transportation prior to performing injections or further handling procedures. Reconstitution should take place no longer than 4 hours post-irradiation to ensure mice are not left immunocompromised for too long.

5.1.4 Following a reconstitution/graft procedure, mice should be monitored closely for at least 15 minutes before returning to their group housing. Mice must be checked for bleeding or swelling from the tail vein injection site, which can be relieved with gentle digital pressure over the site, or signs of an adverse reaction to the graft such as breathing distress or shock, which would require immediate euthanasia.

5.2 Post irradiation monitoring

5.2.1 A tiered system should be utilised to enable efficient and effective group surveillance, but with scope to direct more thorough monitoring toward animals identified as most at risk.

5.2.2 Each room housing animals should have its own record sheet that includes a list of intervention criteria and the scoring system. Information for each individual must list animal number, start weight and note the 10% weight loss value for rapid identification of this intervention point.
5.2.3 A sample of the Visual Inspection List and Intervention Criteria Sheet (Clinical Inspection List) specific to this type of work is provided at the end of this document in Appendix I.

5.3 Visual Inspection

5.3.1 Each visual inspection is carried out with the mice remaining in situ so they can be observed interacting as a group and individually. Any mice with an abnormal result are identified for further clinical inspection.

5.3.2 Appearance: Mice should have a smooth coat (no ruffling of fur), regular breathing and absence of hunching or back arching.

5.3.3 Eyelids: Eyelids should be 75% open for normal mice, though up to 50% open is acceptable as a mild sign. (See Appendix II for further details and photo guide)

5.3.4 Body Condition: Well-conditioned with vertebrae and dorsal pelvis visible but not prominent. This can be further assessed by palpation if required and can be felt by slight pressure. This is BC3 on the Body Condition Score Chart (See Appendix I)

5.3.5 Behaviour: Mice should be alert, interacting with cage mates and moving around the cage.

5.4 Clinical Inspection

5.4.1 Mice are removed from their cage and assessed according to the Intervention Criteria Sheet (See Appendix I).

5.4.2 Minimum recommended monitoring protocol:

Week 1; Day 1-7
- Visual inspection once per day (Appearance, behaviour, body condition score, eyes)
- Weight recorded for all mice at least twice per week
- Visual inspection increased to twice daily and weight recorded once daily if any one of the following signs observed:
  - ≥10% loss of baseline body weight
  - 1 or more moderate signs are identified from Intervention Criteria table
  - Visual inspection finds Body Condition 2 (BC2)

Weeks 2 and 3; Day 8-21
- Visual inspection twice per week (Appearance, behaviour, body condition score, eyes)
- Weight recorded for all mice at least twice per week
- Visual inspection increased to twice daily and weight recorded once daily if any one of the following signs observed:
  - ≥10% loss of baseline body weight
  - 1 or more moderate signs are identified from Intervention Criteria table
  - Visual inspection finds Body Condition 2 (BC2)

Week 4 and beyond; Day 22+
- Visual inspection once a week (Appearance, behaviour, body condition score, eyes)
- Weight recorded for all mice once a week
  - Euthanasia is indicated if mice have not returned to their baseline body weight by day 25
- Visual inspection increased to once daily and weight recorded once daily if any one of the following signs observed:
  - ≥10% loss of baseline body weight
  - 1 or more moderate signs are identified from Intervention Criteria table
  - Visual inspection finds Body Condition 2 (BC2)

5.5 Intervention points and actions required

5.5.1 10-15% loss of baseline body weight
If not already offered, ensure mash is available for mice to eat (at floor level). Ideally, a heat pad should be placed under half of the cage to allow mice to move freely between the heated and non-heated ends while awaiting the AWO. Hydration should be provided as in 5.5.2.

5.5.2 Hydration
For scores of moderate, HydroGel (Clear H₂O®) should be offered initially. At the second daily check if
hydration has not improved then 0.5ml warm, sterile saline or Hartmann's solution should be injected subcutaneously using a 26g needle over a maximum of two sites.

5.5.3 Diarrhoea without blood
Where loose faeces are noted, HydroGel® should be offered pre-emptively to assist hydration as fluid losses will be higher. Close attention should be paid to hydration if diarrhoea persists or blood develops.

5.6 Humane endpoints
5.6.1 Where the health and wellbeing of the animal is in question and the actions taken at earlier intervention points have been insufficient to alleviate problems, guidelines for humane endpoints are to be adhered to. This is to ensure the mice do not suffer and their welfare is maintained.

5.6.2 Mice who have successfully reconstituted should be clinically and visibly improved by days 14-21, though some fluctuation in weight may be expected over this time. Complete immunocompetence is not expected until 6-8 weeks post reconstitution.

5.6.3 Animals that have received a lethal dose of radiation and unsuccessfully reconstituted will rapidly deteriorate. Euthanasia is required immediately if 1 or more 'Severe' signs are observed or if they have not recovered to their initial baseline bodyweight by day 25.

Euthanasia will also be indicated if these humane endpoint criteria are reached at any point:
- Weight loss ≥20%
- Inability to eat
- Laboured breathing (increased effort and respiratory rate)
- Blood in stools

6. ADDITIONAL INFORMATION

6.1 During the period when immune reconstitution is occurring, animals are immunocompromised and at a greater risk of infection. Strict aseptic technique should be used when handling these animals. The use of individually ventilated caging and class 11 safety cabinets during handling are strongly recommended.

6.2 Provision of antibiotic medication in drinking water is common practice in a laboratory setting due to its cost effectiveness and ease of delivery to multiple animals. Limitations of this method include variability in volume consumed by individuals and thus dose ingested, of particular concern in already debilitated animals that may not be voluntarily drinking ‘normal’ assumed levels. Product stability and efficacy may be unknown, especially if it has not been produced in and/or standardised by a pharmaceutical laboratory. The benefits and limitations of antibiotic use should be decided on an individual project basis. The AWO should be consulted for further information on appropriate antibiotic selection and use.

7. ENFORCEABLE REQUIREMENTS

7.1 Reconstitution of irradiated mice is required unless specific justification is provided in the application and approved by the AEC.

7.2 Irradiation procedures must only be administered by trained personnel and within an approved, prescribed facility. A prescribed radiation facility is a facility that is prescribed in Regulation 6 of the Australian Radiation Protection and Nuclear Safety Regulations 1999 (the Regulations).

7.3 Adherence to minimum monitoring frequencies as described in Section 4.3 and Section 5. Additional monitoring may be required for short term studies or depending on the nature of the research.

7.4 Adherence to humane endpoints to prevent unnecessary suffering of mice; Mice with severe symptoms must be killed on the same day.

8. EXEMPTIONS

Where adherence to this Standard conflicts with proposed work, the University's AECs may grant exemptions to all or part of the Standard. To seek exemption, applications should clearly outline how the proposed work deviates from the Standard, and justify the need for this. Before seeking exemption, it is recommended that you consult with the University's AWO.
9. **UNEXPECTED ADVERSE INCIDENTS**

An unexpected adverse event is any event, which impacts negatively on the wellbeing of animals, and which was not anticipated, or has occurred at a frequency or severity in excess of what was anticipated in line with the AEC approval. This can be a single or cumulative event, and will normally involve unexpected mortality, morbidity or injury. Anyone identifying an unexpected adverse event must act to remove and/or minimise any immediate risk to animals. Immediately thereafter, the University’s AWO and relevant Animal Facility Manager must be notified of the event. The AWO will advise researchers of the appropriate response.

10. **GLOSSARY**

<table>
<thead>
<tr>
<th>Scientific Term</th>
<th>Lay Description</th>
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</thead>
<tbody>
<tr>
<td>Myeloablation</td>
<td>The severe or complete depletion of bone marrow cells</td>
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<tr>
<td>Lethal dose</td>
<td>The irradiation dose likely to cause death in a certain percentage of the test animals, usually 50%.</td>
</tr>
<tr>
<td>Sublethal dose</td>
<td>A dose of gamma radiation that is not large enough to cause death</td>
</tr>
<tr>
<td>Bacterial Translocation</td>
<td>When the wall of the intestinal tract is weakened (as occurs in irradiated animals), the blood vessels in the area become more permeable. Bacteria that are normally contained in the gut are able to move through this compromised layer and travel in the blood stream to other sites in the body, typically the liver, kidneys, lungs or brain.</td>
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<tr>
<td>Bone marrow</td>
<td>Refers to the group of immune system cells and their precursor forms that reside and multiply in the bone marrow.</td>
</tr>
<tr>
<td>Reconstitution</td>
<td>To return to a former state. Following a dose of irradiation, host bone marrow is destroyed. Donor cells are then injected into the tail vein and these repopulate the host’s bone marrow. If successful, after 21 days, the host is now considered to have ‘reconstituted’ bone marrow.</td>
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<tr>
<td>Immunocompetent</td>
<td>An animal with a fully functioning immune system, including the cells from the bone marrow. There is a delay between when reconstitution occurs (the cells are present) and developing immunocompetence (the cells are able to function optimally).</td>
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<tr>
<td>Graft</td>
<td>Transplant</td>
</tr>
<tr>
<td>Ill-thrift</td>
<td>Not thriving physically</td>
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<tr>
<td>Inappetence</td>
<td>Lack of appetite</td>
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<tr>
<td>Moribund</td>
<td>At the point of death</td>
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<tr>
<td>In situ</td>
<td>In the original position, undisturbed. In this case, mice are left in the cage and not picked up.</td>
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<tr>
<td>Hartmann’s solution</td>
<td>Type of electrolyte balanced medical fluids commonly used in small animal medicine (also called “Lactated Ringer’s Solution” or LRS)</td>
</tr>
</tbody>
</table>

11. **REFERENCES & RESOURCES**

The following source material contributed to the development of this Standard:

APPENDIX I:

Visual Inspection List

Mice are left in their cage and visually assessed.

1. **Appearance**: Mice should have a smooth coat (no ruffling of fur), regular breathing and absence of hunching or back arching.

2. **Body Condition**: Well-conditioned with vertebrae and dorsal pelvis palpable under slight pressure. This can be further assessed by palpation if required and will be felt by slight pressure. This is BC3 on the Body Condition Score Chart.

3. **Behaviour**: Mice should be alert, interacting with cage mates and moving around the cage.

4. **Eyes**: Eyelids should be 75% open for normal mice, though up to 50% open is acceptable as a mild sign.

In the event of mice showing signs of ill health or not matching the criteria above, the assessor is referred to the Intervention Criteria Sheet (Clinical Inspection List) for increased monitoring requirements.

Mice with severe symptoms must be killed on the same day and not left overnight or over the weekend. If required, the Animal Welfare Officer (AWO) or Animal Facility Manager (AFM) should be contacted for advice.

**BODY CONDITION SCORE SHEET**

| BC 1 | Mouse is emaciated.  
| - Skeletal structure extremely prominent; little or no flesh cover.  
| - Vertebrae distinctly segmented. |
| BC 2 | Mouse is underconditioned.  
| - Segmentation of vertebral column evident.  
| - Dorsal pelvic bones are readily palpable. |
| BC 3 | Mouse is well-conditioned.  
| - Vertebrae and dorsal pelvis not prominent; palpable with slight pressure. |
| BC 4 | Mouse is overconditioned.  
| - Spine is a continuous column.  
| - Vertebrae palpable only with firm pressure. |
| BC 5 | Mouse is obese.  
| - Mouse is smooth and bulky.  
| - Bone structure disappears under flesh and subcutaneous fat.  
|  

A “+” or a “−” can be added to the body condition score if additional increments are necessary (i.e. ...2+, 2−, ...)

### Intervention Criteria Sheet - Clinical Inspection List

**Criteria are based on the severity of signs, as classified by the table below:**

<table>
<thead>
<tr>
<th>Observations (see Severity Table below)</th>
<th>Action required</th>
</tr>
</thead>
<tbody>
<tr>
<td>No/Mild signs</td>
<td>In week 1: Visual inspection once daily, weight twice weekly. In Week 2 and 3: Visual inspection and weight twice weekly. In week 4+: Visual inspection and weight once weekly.</td>
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<tr>
<td>1 or more “Moderate” signs (see below)</td>
<td>Visual inspection twice daily, weight once daily</td>
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<tr>
<td>1 or more “Severe” signs (see below)</td>
<td>Euthanasia</td>
</tr>
</tbody>
</table>

#### Severity Table

<table>
<thead>
<tr>
<th>Non-specific sign(s):</th>
<th>No/Mild</th>
<th>Moderate</th>
<th>Severe</th>
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<tbody>
<tr>
<td>1. Appearance</td>
<td>Smooth, glossy coat Minor fight wounds (separate mice if fighting) Slight to moderate ruffling of fur and/or back arching. Severe ruffling of fur and/or hunching. Major fight wounds. Rapid, shallow breathing.</td>
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<td>2. Body condition</td>
<td>Mouse is well-conditioned i.e. BC3 Mouse is under-conditioned i.e. BC2 Mouse is under-conditioned or emaciated i.e. BC1 or BC2, Kill mice if weight loss ≥20%.</td>
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<td>3. Behaviour</td>
<td>Normal behaviour (no sign of distress) Subdued but responsive, decreased interaction with peers Minimally responsive or unresponsive to activity and provocation</td>
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<td>4. Eyes (eyelids open)</td>
<td>50-75% open= normal or mild 25-49% open &lt;25% open</td>
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<td>5. Anaemia</td>
<td>Foot pads appear normal colour Footpad colour is paler compared to normal Footpad colour is not improved after 2 days.</td>
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<td>6. Intestinal bleeding</td>
<td>Normal (items 1-3 above) Reduced movement; increased respiration rate or effort Obtunded, obvious respiratory distress</td>
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<td>7. Diarrhoea*</td>
<td>Normal formed faeces Moist faeces, can be runny* Diarrhoea, mucous and blood streaks (bright or dark red)</td>
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<tr>
<td>8. Hydration**</td>
<td>Normal; bright eyes, no skin tent Decreased skin elasticity, dull eyes** Skin tenting and/or sunken eyes</td>
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<td>9. Injection site inflation</td>
<td>No visible reaction Mild to moderate redness or swelling Site of injection cracked, bleeding or has discharge</td>
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<td>10. Other signs</td>
<td>Seek AFM/AWO advice re: appropriate action or euthanise if animal is in moderate or severe pain or distress</td>
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**Specific actions:**

*7. Diarrhoea (without blood)- If loose faeces are noted, HydroGel® should be offered pre-emptively to assist hydration as fluid losses will be higher. Close attention should be paid to hydration if diarrhoea persists or blood develops.*

**8. Hydration-** For scores of moderate HydroGel® should be offered initially. At the second daily check 0.5ml warm, sterile saline should be injected subcutaneously using a 26g needle over a maximum of two sites if hydration is no better.
APPENDIX II: Appearance of eyes for grading in Visual Inspection

Figure 1. Scoring of eye appearance according to cageside observations

Images in figure 1 depict the eyes of mice;
Normal eyes, eyelids open 75% or more
Eyes open 50-75% (Mild sign)
Eyelids open 25-49% (Moderate sign)
Eyelids open ≤25% (Severe sign)

APPENDIX III:
Monitoring sheet

for (Ethics Application #):

Principal Investigator:

Secondary contact person:

Cage or animal number(s) covered by this sheet:

Agreed frequency of monitoring:

Intervention: will be as per Intervention Criteria Sheet attached.

<table>
<thead>
<tr>
<th>Date:</th>
<th>Exp day #:</th>
<th>Time:</th>
<th>Animal no.:</th>
<th>Body-weight (g):</th>
<th>Normal</th>
<th>Abnormal appearance (coat condition, eyes &amp; posture)</th>
<th>Abnormal behaviour</th>
<th>Body Condition (BC1-5)</th>
<th>Weight loss (% of starting weight)</th>
<th>Other signs (describe)</th>
<th>Comments/Procedures performed/Actions taken (include Fate):</th>
<th>Initials:</th>
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Monitoring mice post-irradiation [Version 1]