# Institutional Animal Care and Use Committee (IACUC) Policies

## Table of Contents

<table>
<thead>
<tr>
<th>Policy</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(Tumor Models)</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>(Overcrowding)</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>(Humane Endpoints and Animal Monitoring)</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>(Rodent Euthanasia)</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>(Body Weight Loss)</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td>(Cell Line Usage and Rodent-Derived Biological Products)</td>
<td>27</td>
</tr>
<tr>
<td>7</td>
<td>(Experimental Allergic Encephalitis (EAE) in Mice)</td>
<td>29</td>
</tr>
<tr>
<td>8</td>
<td>(Tissue Collection for Genotyping of Mice and Rats)</td>
<td>32</td>
</tr>
<tr>
<td>9</td>
<td>(Mouse Toe Clipping for Identification)</td>
<td>35</td>
</tr>
<tr>
<td>10</td>
<td>(Guidelines for Rodent Surgery)</td>
<td>37</td>
</tr>
<tr>
<td>11</td>
<td>(Use of Drugs and Expired Materials)</td>
<td>46</td>
</tr>
<tr>
<td>12</td>
<td>(Use of Recording Devices)</td>
<td>50</td>
</tr>
<tr>
<td>13</td>
<td>(Q Fever and Zoonotic Disease Prevention, Sheep)</td>
<td>52</td>
</tr>
<tr>
<td>14</td>
<td>(Identification of Rodents)</td>
<td>54</td>
</tr>
<tr>
<td>15</td>
<td>(Mouse Total Body Irradiation)</td>
<td>57</td>
</tr>
<tr>
<td>16</td>
<td>(Social Housing and Environmental Enrichment)</td>
<td>61</td>
</tr>
<tr>
<td>17</td>
<td>(Personal Protective Equipment Requirement for In Vivo Studies)</td>
<td>62</td>
</tr>
<tr>
<td>18</td>
<td>(Monoclonal Antibody Production and Ascites)</td>
<td>63</td>
</tr>
<tr>
<td>19</td>
<td>(Fluid Administration and Collection in Rodents)</td>
<td>66</td>
</tr>
<tr>
<td>20</td>
<td>(Use of Adjuvants)</td>
<td>72</td>
</tr>
<tr>
<td>21</td>
<td>(PI Responsibilities Regarding Animal Care and Use)</td>
<td>74</td>
</tr>
<tr>
<td>22</td>
<td>(Animal Welfare Concern Reporting/Whistleblower)</td>
<td>77</td>
</tr>
</tbody>
</table>
Policy 1 – Tumor Models
Version 2.0
Approval Date: 1/22/07, 1/16/13

Purpose – The purpose of this policy is to provide information about common tumor models in rodents, monitoring of animals in cancer studies, and limits regarding maximum allowable size of tumors.

Background - This policy includes: 1) tumors induced by injecting cells/tumor fragments into animals (eg. xenograft or allograft models), 2) spontaneous, naturally occurring tumors (eg. geriatric tumors or thymoma in NOD-SCID mice), and 3) chemically induced tumors in mutant mice (eg. Cre-lox mice).

abbreviations:
BCS = body condition score

Policy –

1. Pathogen testing of tumor cell lines: All rodent or human cells/tumors injected into rodents must be tested for infectious agents according to Policy 6 (Use of Cell Lines).

2. Tumor injection site: Tumor injection site(s) should be chosen so not to interfere with normal bodily functions such as walking, eating, drinking, defecation, or urination. The recommended site is on the flank, half-way between elbow and iliac crest (see below). Sites involving sensory functions, such as the eye, should be avoided. Intramuscular (IM) implantation should be avoided as growing tumor causes muscle distention and pain. Use of inhalation anesthetic prior to cell injections is recommended for safety of personnel. Tumor injection site(s) must be disinfected using 70% alcohol before injection. Refer to Policy 19 (Fluid Administration and Collection) for determining the appropriate volume and needle size to be used for each species.

Recommended site for subcutaneous injection (SC) of cells/tumors

3. Pieces of tumors: Usually delivered subcutaneously through a large-bore needle called a trocar. Because of the large diameter of a trocar (≤16ga), more than momentary pain is associated with their use. Therefore, all procedures involving trocars are considered minor survival surgery by the IACUC. Animals MUST be under general anesthesia or have a local anesthetic agent applied at the trocar site for these procedures. Trocars will cause more damage to the skin compared to smaller gauge hypodermic needles, and skin closure (suture, staples, wound clips, surgical glue) may be
needed post-injection. Additionally, animals should be provided with analgesia for at least 12hrs postoperatively.

4. **Number of tumors injected into each animal:** Maximum of two tumor injections permitted for each animal.

5. **Frequency of monitoring:** All animals involved in tumor studies must be monitored for tumor size, pain, and distress at least three times per week by qualified laboratory personnel. Animals that are approaching humane endpoints (i.e. tumor diameter ≥1.0cm (mice) or BCS≤2) must be monitored daily, including holidays and weekends.
   a. **Tumor measurement:** tumor size must be determined at least weekly.
   b. **Body weight measurement:** BSC is recommended and must be performed at least weekly.
      Measuring animal body weight can be misleading as it includes tumor weight (refer to IACUC policy #5, Weight Loss).

6. **Analgesics:** Any animal determined to be in pain or distress (evaluated by appearance, behavior, or clinical signs) must be provided with analgesics. Withholding analgesics must be scientifically justified and approved by the IACUC prior to the initiation of the study.

7. **Euthanasia:** The following conditions require euthanasia:
   a. Animals develop significant tumors unrelated to the experimental studies
   b. Tumors exceed maximum allowable size:
      i. Mouse: single tumor with diameter of ≥1.5cm, two tumors with diameter of ≥1.0cm (each)
      ii. Rat: single tumor with diameter of ≥3.0cm, two tumors with diameter of ≥2.0cm (each)
   c. Volume ≥ 1700mm³ (mouse) or ≥3400mm³ (rat); use the following formulas to calculate volume (based on tumor shape):
      i. **Spherical:** \((\text{width}^2 * \text{length}) / 2\) [citation 3]
      ii. **Ellipsoid:** \(\pi/6 * (\text{length})*\text{(width})*\text{(height)}\) [citation 4]
   d. Tumors interfere with walking, eating, drinking, urination, or defeation
   e. Tumors result in BCS ≤1.5 (Policy 5, Weight Loss)
   f. Ulceration, infection or necrosis of tumors
   g. Animals show clinical symptoms described in IACUC Policy 3 (Humane Endpoints)
   h. Any animal evaluated by appearance, behavior, or clinical signs to be in pain or distress (unless pre-approved by IACUC)

   **Any exceptions to this policy must have prior IACUC approval**

References -

   [http://dels.nas.edu/ilar_n/ilarjournal/41_2/CancerResearch.shtml](http://dels.nas.edu/ilar_n/ilarjournal/41_2/CancerResearch.shtml)
   [http://www.biomedcentral.com/content/pdf/1471-2342-8-16.pdf](http://www.biomedcentral.com/content/pdf/1471-2342-8-16.pdf)
Policy 2 – Overcrowding
Version 2.0
Approval Date: 1/22/07, 1/16/13

Purpose – The purpose of this policy is to provide investigators guidelines for the appropriate stocking density of mice and rats (based on the Guide’s requirements) and describe the notification and penalty system regarding overcrowded cages.

Background – RWJMS uses standard “shoelace” style caging (plastic tub with lid) for the majority of rodents. The standard mouse cage at RWJMS is 7.5W x 11.75D x 5H (inches, either ~67in² or ~75in² useable floor space depending on specific cage type) and can hold a maximum of 5 adult mice (25g each) OR one breeding pair (one male and one female) with a litter. Obese mice may require more space as determined by the veterinary staff (appendix I). The standard rat cage at RWJMS is 10.1W x 19D x 8H (inches, ~143in² useable floor space) and can hold a maximum of 3 adult rats (400g each) OR one breeding pair (one male and one female) with a litter. Most mouse and rat strains must be weaned and placed into appropriate housing groups at 21 days of age (exceptions included in appendix II). However, special arrangements can be made for alternative weaning times, following discussion with the veterinary staff.

Policy –

Overcrowded cages include:

1. More than 5 adult mice (25g each) in a standard cage; this includes animals awaiting euthanasia (in animal holding rooms or placed in the necropsy room).

2. More than 3 adult rats (400g each) in a standard cage; this includes animals awaiting euthanasia (in animal holding rooms or placed in the necropsy room).

3. Two (or more) litters in one cage: if trio breeding (one male and two females), pregnant females MUST be placed in separate cages prior to parturition.

4. All litters beyond weaning age (21 days): refer to appendix II for exceptions regarding certain mouse strains.

Notification of overcrowded cage(s):

1. Overcrowded cage(s) will be marked with an orange "Overcrowded Cage" notification card by animal care or veterinary staff and the PI (or lab designee) will be notified via an e-mail by the veterinary staff. PIs will be charged $5 for EACH boxed marked as ‘Overcrowded’.

2. Severely overcrowded cages (15+ animals) must be separated within 24hrs of notification (or sooner in the interest of animal welfare, based on the discretion of the veterinary staff).
Removal of “Overcrowded Cage” notification card(s), prior to correction of overcrowding, will be immediately reported to the IACUC

Overdue cages:
If the PI (or lab designee) does not correct overcrowded cage(s) within the 72 hour (3d) time period, veterinary staff will separate the animals into the appropriate number of cages (eg. 15 newly weaned mice divided into 3 cages). The researcher will be billed $10 for each cage necessary to satisfy the overcrowding policy. If the PI has more than three incidents in a 30 day period, the violation(s) will be reported to the IACUC.

Any exceptions to this policy must have prior IACUC approval

Appendix I - Recommended minimum space for commonly used laboratory rodents housed in groups [the Guide 8th Ed., page 62, table 3.2]

<table>
<thead>
<tr>
<th>Animals</th>
<th>Weight (g)</th>
<th>Floor Area/Animal,(^a) (\text{in}^2) (cm(^2))</th>
<th>Height,(^b) in (cm)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice in Groups (^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10</td>
<td></td>
<td>6 (38.7)</td>
<td>5 (12.7)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td>Up to 15</td>
<td></td>
<td>8 (51.6)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td>Up to 25</td>
<td></td>
<td>12 (77.4)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td>&gt;25</td>
<td></td>
<td>≥15 (≥96.7)</td>
<td>5 (12.7)</td>
<td></td>
</tr>
<tr>
<td>Female + Litter (mouse)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td></td>
<td>17 (109.6)</td>
<td>7 (17.8)</td>
<td>Larger animals may require more space to meet the performance standards.</td>
</tr>
<tr>
<td>Up to 200</td>
<td></td>
<td>23 (148.35)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Up to 300</td>
<td></td>
<td>29 (187.05)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Up to 400</td>
<td></td>
<td>40 (258.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Up to 500</td>
<td></td>
<td>60 (387.0)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>&gt;500</td>
<td></td>
<td>≥70 (≥451.5)</td>
<td>7 (17.8)</td>
<td></td>
</tr>
<tr>
<td>Female + Litter (rats)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;100</td>
<td></td>
<td>124 (800.0)</td>
<td>7 (17.8)</td>
<td>Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.(^d)</td>
</tr>
</tbody>
</table>

\(^a\) Singly housed animals and small groups may require more than the applicable multiple of the indicated floor space per animal.

\(^b\) From cage floor to cage top.

\(^c\) Consideration should be given to the growth characteristics of the stock or strain as well as the sex of the animal. Weight gain may be sufficiently rapid that it may be preferable to provide greater space in anticipation of the animal’s future size. In addition, juvenile rodents are highly active and show increased play behavior.

\(^d\) Other breeding configurations may require more space and will depend on considerations such as number of adults and litters, and size and age of litters.
Other considerations may include culling of litters or separation of litters from the breeding group, as well as other methods of more intensive management of available space to allow for the safety and well-being of the breeding group. Sufficient space should be allocated for mothers with litters to allow the pups to develop to weaning without detrimental effects for the mother or the litter.

Appendix II – Weaning ages for commonly used strains of mice (from Jackson Laboratories)

<table>
<thead>
<tr>
<th>Stock #</th>
<th>Strain Name</th>
<th>Group</th>
<th>Wean Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>000457</td>
<td>B10.RIII H2&lt; r &gt; H2 T18&lt; b &gt;/ ( 71NS )Sn</td>
<td>Congenic</td>
<td>4</td>
</tr>
<tr>
<td>000461</td>
<td>B10.D2 HC&lt;0&gt; H2&lt;D&gt; H2 T18 &lt;c&gt;/OSnJ</td>
<td>Congenic</td>
<td>4</td>
</tr>
<tr>
<td>000463</td>
<td>B10.D2 HC&lt;1&gt; H2&lt;D&gt; H2 T18 &lt;c&gt;/NSnJ</td>
<td>Congenic</td>
<td>4</td>
</tr>
<tr>
<td>000465</td>
<td>B10.BR H2&lt;k&gt; H2-T18&lt;a&gt;/SgSnJ</td>
<td>Congenic</td>
<td>4</td>
</tr>
<tr>
<td>000476</td>
<td>C57BL/10ScSn</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000486</td>
<td>MRL/MpJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000635</td>
<td>C3H/HeOuJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000645</td>
<td>A/HeJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000646</td>
<td>A/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000647</td>
<td>A/WySnJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000648</td>
<td>AKR/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000651</td>
<td>BALB/cJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000653</td>
<td>BUB/BnJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000654</td>
<td>CBA/CaJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000656</td>
<td>CBA/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000657</td>
<td>CE/J</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000658</td>
<td>C3HeB/FeJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000659</td>
<td>C3H/HeJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000661</td>
<td>C3H/HeSnJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000662</td>
<td>C57BLK5/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000664</td>
<td>C57BL/6J</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000665</td>
<td>C57BL/10J</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000666</td>
<td>C57BL/10SnJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000667</td>
<td>C57BR/CdJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000668</td>
<td>C57L/J</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000669</td>
<td>C58/J</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000670</td>
<td>DBA/1J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000671</td>
<td>DBA/2J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000674</td>
<td>I/LnJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000676</td>
<td>LP/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000679</td>
<td>P/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000680</td>
<td>PL/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000682</td>
<td>RF/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000683</td>
<td>RIIS/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000684</td>
<td>NZB/B1NJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000686</td>
<td>SJL/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>ID</td>
<td>Strain Name</td>
<td>Type</td>
<td>Category</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------</td>
<td>---------</td>
<td>----------</td>
</tr>
<tr>
<td>000687</td>
<td>SM/J</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>000689</td>
<td>SWR/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000690</td>
<td>129P3/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000691</td>
<td>129X1/SvJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000726</td>
<td>RBF/DnJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>000928</td>
<td>CAST /Ei</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>001011</td>
<td>CBA/CaHN Btk &lt; Xid &gt;/J</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>001026</td>
<td>BALB/cByJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>001058</td>
<td>NZW/LacJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>001060</td>
<td>B6.C-H2&lt;bm1&gt;/ByJ</td>
<td>Congenic</td>
<td>4</td>
</tr>
<tr>
<td>001137</td>
<td>129P1/ReJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>001139</td>
<td>C57BL/6ByJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>001140</td>
<td>DBA/1LacJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>001143</td>
<td>CBA/CaGnLe</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>001162</td>
<td>B6.C H2 &lt; bm12 &gt;/KhEg</td>
<td>Congenic</td>
<td>3</td>
</tr>
<tr>
<td>001165</td>
<td>BALB/C-H2&lt; dm2 &gt;/KhEg</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>001317</td>
<td>C57BL/6J Igh &lt;a&gt; Thy1 &lt;a&gt; Gpi1 &lt;a&gt;</td>
<td>Congenic</td>
<td>3</td>
</tr>
<tr>
<td>001800</td>
<td>FVB/NJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>001976</td>
<td>NOD/LTJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>002014</td>
<td>B6.SJL Ptprc &lt;a&gt; Pep3 &lt;b&gt;/BoyJ</td>
<td>Congenic</td>
<td>3</td>
</tr>
<tr>
<td>002024</td>
<td>B10.D1 H2 &lt; q &gt;/SgJ</td>
<td>Congenic</td>
<td>4</td>
</tr>
<tr>
<td>002050</td>
<td>NOR/LTJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>002065</td>
<td>129T2 /SvEmSj</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>002105</td>
<td>NZO /HIJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>002106</td>
<td>KK /HIJ</td>
<td>INBRED</td>
<td>4</td>
</tr>
<tr>
<td>002282</td>
<td>BTBR T &lt;+&gt; tf /tf</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>002423</td>
<td>NON/LTJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>002448</td>
<td>129S1 / SvImJ</td>
<td>INBRED</td>
<td>3</td>
</tr>
<tr>
<td>004483</td>
<td>NOD,NON Thy1 &lt;a&gt; / 1LtJ</td>
<td>Congenic</td>
<td>3</td>
</tr>
</tbody>
</table>

References -


Purpose – The purpose of this policy is to provide investigators with guidelines pertaining to animals that are nearing death, relating to monitoring of such animals and when euthanasia is required.

Background –

“Animals that would otherwise suffer severe or chronic pain or distress that cannot be relieved should be painlessly killed at the end of the procedure or, if appropriate, during the procedure.” - OLAW [1]

“Studies that may result in severe or chronic pain or significant alterations in the animal’s ability to maintain normal physiology, or adequately respond to stressors, should include descriptions of appropriate humane endpoints or provide science-based justification for not using a particular, commonly accepted humane endpoint.” - the Guide [2]

Death as an Endpoint - The continuation of a study until an animal dies is almost never acceptable. Strong scientific justification is required for such a study.

Policy -

Selected Criteria For Euthanasia: [3, 4, and 5]

- Weight loss ≥15% within one week (considered rapid weight loss)
- Weight loss ≥ 20% over any time period (progressing to an emaciated state)
- Body condition scoring (BCS) <1.5 (refer to Policy 5)
- Lesions (such ulcerative dermatitis) covering ≥10% of the skin (see Table 1)
- Rough hair coat, hunched posture, distended abdomen, or lethargy; especially if debilitating or prolonged (≥3 days)
- Diarrhea; especially if debilitating or prolonged (≥3 days)
- Coughing, rales, wheezing, or nasal discharge
- Distinct icterus (yellow skin) and/or anemia (pale skin)
- Rapid growth of mass (or masses), or clinical signs of neoplasia not related to study (see Policy 1 for cancer models)
- Central nervous system signs such as head tilt, tremors, spasticity, seizures, circling, or paralysis/paresis, especially if associated with anorexia
- Frank bleeding from any orifice
- Significant hypothermia
- Markedly discolored urine, polyuria, or anuria
- Persistent self-induced trauma
- Lesions interfering with eating or drinking
- Clinical signs of suspected infectious disease requiring necropsy for diagnosis
- Other clinical signs as judged by the Veterinary Staff to be indicative of moribund condition

**Frequency of Monitoring:** Research Staff must monitor all animals at least 3 times a week. Animals approaching humane endpoints must be monitored daily including weekends and holidays (or more), as according to the IACUC approved protocol.

**Geriatric animals:** Geriatric mice (>16 months of age) must be monitored for tooth loss weekly. If tooth loss is observed, animals must be provided with softened food and overgrown incisors must be trimmed every 1-2 weeks (recorded on blue treatment card). Because these animals are prone to tumors and organ failure, Policies 1, 3, and 5 must also be followed.

**Table 1 - Body surface area (BSA) of mice for evaluating significance of skin lesions [6]**

<table>
<thead>
<tr>
<th>BW (gm)</th>
<th>Body surface area (cm²)</th>
<th>10% BSA (cm²)</th>
<th>Diameter of circle covering 10% BSA, (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>65</td>
<td>6.5</td>
<td>2.8</td>
</tr>
<tr>
<td>25</td>
<td>75</td>
<td>7.5</td>
<td>3.1</td>
</tr>
<tr>
<td>30</td>
<td>84</td>
<td>8.4</td>
<td>3.3</td>
</tr>
<tr>
<td>35</td>
<td>95</td>
<td>9.5</td>
<td>3.5</td>
</tr>
<tr>
<td>40</td>
<td>102</td>
<td>10.2</td>
<td>3.6</td>
</tr>
</tbody>
</table>

(Meeh coefficient for mouse = 9)

Any exceptions to this policy must have IACUC and Veterinary approval

**References**

   http://www.nal.usda.gov/awic/newsletters/v6n1/v6n1otbl3.htm
Policy 4 – Rodent Euthanasia
Version 2.0
Approval Date: 1/22/07, 3/5/13

Purpose – The purpose of this policy is to provide investigators with acceptable and conditionally methods of euthanasia for various species and ages of rodents, along with methods that are not acceptable.

Background –

“…methods [of euthanasia] should be consistent with the AVMA Guidelines on Euthanasia (AVMA 2007 or later editions).” – the Guide

“Standardized methods of euthanasia that are predictable and controllable should be developed and approved by the Attending Veterinarian and IACUC.” – the Guide

Classification of method of euthanasia: The AVMA categorizes each method of euthanasia as acceptable (methods which consistently produce a humane death when used as the sole means of euthanasia), conditionally acceptable (methods which by the nature of the technique or because of greater potential for operator error or safety hazards might not consistently produce humane death or are methods not well documented in the scientific literature) or unacceptable (methods deemed inhumane under any conditions or that the panel found posed a substantial risk to the human applying the technique).

Policy –

Euthanasia quick reference chart:

<table>
<thead>
<tr>
<th>age</th>
<th>accepted</th>
<th>conditionally accepted</th>
<th>not accepted</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>early embryos (E-1 to E15)</td>
<td>death of dam is sufficient, no other methods are needed</td>
<td>N/A</td>
<td>N/A</td>
<td>dam needs to be euthanized using accepted methods</td>
</tr>
<tr>
<td>late embryos to neonates (E16-P-9)</td>
<td>decapitation, cervical dislocation, chemical injection</td>
<td>N/A</td>
<td>CO₂ inhalation, hypothermia</td>
<td>animals are resistant to hypoxia at this age, CO₂ inhalation is not accepted</td>
</tr>
<tr>
<td>P-10 to adult</td>
<td>Gradual exposure to CO₂, anesthetic overdose (chemical injection, inhalation)</td>
<td>Cervical dislocation, decapitation, need IACUC approval</td>
<td>CO₂ from dry ice, hypothermia, smothering, blow to head</td>
<td>inhalation method must be followed by a physical euthanasia method</td>
</tr>
</tbody>
</table>

Overview:

Section 1: General Information Regarding Rodent Euthanasia
Section 2: Euthanasia of Adult Rodents (≥10d)
Section 3: Euthanasia of Rodent Fetuses and Neonates (E16-P9)
Section 4: Effects of Various Methods of Euthanasia on Rodent Physiology
SECTION 1: General Information

Euthanasia is the act of humanely killing animals by methods that induce rapid unconsciousness and death without pain or distress. Public Health Service policy requires that the Institutional Animal Care and Use Committees (IACUC) determines that methods of euthanasia utilized in research proposals are consistent with the recommendations of the 2013 American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (1).

The criteria used as the basis for the AVMA’s recommendations include:

1. Minimum pain, distress, anxiety or apprehension
2. Minimum delay until unconsciousness
3. Reliability and irreversibility
4. Safety of personnel; emotional effect on personnel
5. Species and age limitations

IACUC approval of any deviation(s) from the 2013 AVMA Guidelines must be project-specific and include critical review of assertions of scientific necessity. If conditionally acceptable techniques are planned, they must be scientifically justified and approved by the IACUC prior to implementing.

Euthanasia must be performed in a compassionate manner to avoid animal distress. Depending on the species involved, animals being euthanized may vocalize, release pheromones or behave in a manner, which may be distressing to other animals. For these reasons, animals may not be euthanized while crowded or in the presence of animals not being euthanized.

Additional required practices to ensure death in rodents - Death must be verified after euthanasia and prior to disposal. Confirmation of death is achieved by absence of respiration, heartbeat and toe/tail pinch reflexes. However, the assessment of heart beat and respiratory pattern can be very difficult in rodents due to their small size. Consequently, these criteria may be difficult to apply to these species and there is a risk of animals recovering. To ensure the irreversibility of inhalant euthanasia, it is required that animals undergo a secondary, physical method of euthanasia (i.e., exsanguination, thoracotomy, cervical dislocation, or decapitation).

Principal Investigators are responsible for ensuring all personnel performing animal euthanasia in an IACUC approved protocol have been properly trained to consistently apply the technique(s) in a humane and effective manner.
SECTION 2: Euthanasia for Rodents ≥10 Days Old (adults)

Inhalant Agents (Acceptable)

When possible, inhaled agents should be administered under conditions where animals are most comfortable (ie for rodents, in their home cage).

Carbon dioxide (2): Carbon dioxide has a rapid depressant, analgesic and anesthetic effect. Carbon dioxide is nonflammable, non-explosive, and poses minimal hazard to personnel when used with properly designed equipment. Because CO₂ is heavier than air, incomplete filling of a chamber may permit animals to climb or raise their heads above the higher concentrations and avoid exposure.

High concentrations of CO₂ may be stressful to some species when awake. Accordingly, pre-filling the chamber is forbidden. Carbon dioxide systems should displace 10-30% of chamber volume per minute. Chambers must not be overcrowded; animals should be able to stand on the floor of the chamber with all four feet and have sufficient space to turn around and perform normal postural adjustments. In this regard, it is important to also consider that mixing unfamiliar or incompatible animals in the same container may also be stressful. Chambers should be emptied and cleaned/disinfected between uses.

Compressed CO₂ gas is the only permitted source of carbon dioxide because the inflow to the chamber can be regulated precisely. Carbon dioxide generated by other methods such as from dry ice, fire extinguishers, or chemical means (i.e., antacids) is forbidden.

Inhalant Anesthetics: Inhalant anesthetics are particularly valuable for euthanasia of smaller animals or for animals in which venipuncture may be difficult. Since the liquid state of most inhalant anesthetics is irritating, animals can only be exposed to vapors and must be prevented from contacting the anesthetic agent in its liquid form. In order of preference, halothane, enflurane, isoflurane, sevoflurane, and desflurane are generally acceptable for euthanasia of small animals (<7 kg). Halogenated anesthetic agents should only be used if they are appropriately scavenged to avoid personnel exposure, i.e., activated charcoal canister, fume hood or exhausted biosafety cabinet class II, type B.

For all inhaled forms of euthanasia, death must be verified after euthanasia and prior to disposal. Unintended recovery must be obviated by the use of appropriate CO₂ concentrations and exposure times or by other means. To ensure the irreversibility of the procedure after apparent death from CO₂, animals must further undergo a physical method of euthanasia (i.e., exsanguination, thoracotomy, cervical dislocation or decapitation; see physical methods listed below).

Inhalant Anesthetics (Conditionally Acceptable)

Ether is irritating to the mucous membranes and poses serious risks associated with its inflammability and explosiveness. Explosions have occurred when animals, euthanatized with ether, were placed in an ordinary (not explosion proof) refrigerator or freezer and when bagged animals were placed in an incinerator. Ether can only be used after IACUC approval in carefully controlled situations in compliance with all applicable safety policies and regulations.
Injectable Pharmaceutical Agents (Acceptable)

Barbiturates: A primary advantage of barbiturates is speed of action, which depends on the dose, concentration, route, and rate of injection. Barbiturates induce euthanasia smoothly, with minimal discomfort to the animal. Intravenous injection is the preferred route of administration, however intraperitoneal injections may be used in situations when intravenous injections would be stressful or impractical. Intracardiac injection can only be used if the animal is heavily sedated, unconscious, or anesthetized. Barbiturates may be administered intraperitoneally and induce rapid, smooth euthanasia with minimal animal discomfort. As with all controlled substances, barbiturate usage requires having appropriate licensure and registration, ensuring secure storage and maintaining accurate drug accountability.

Potassium chloride in conjunction with general anesthesia: Although unacceptable when used in unanesthetized animals, the use of potassium chloride administered intravenously or intracardially in animals under general anesthesia is an acceptable method of euthanasia. It is important for personnel performing this method of euthanasia to be trained and knowledgeable in anesthetic techniques, and competent in assessing anesthetic depth. Administration of potassium chloride requires animals to be in a surgical plane of anesthesia characterized by loss of consciousness, loss of reflex muscle response, and loss of response to noxious stimuli.

Physical Methods (Conditionally Acceptable)

Physical methods of euthanasia when properly used by skilled personnel with well-maintained equipment may result in less fear and anxiety and be more rapid, painless, humane, and practical than other forms of animal euthanasia. Physical methods are not generally allowed as a sole means of euthanasia (exceptions granted from the IACUC), but are required as adjuncts to other agents or methods. Personnel performing physical methods of euthanasia must be trained and monitored for each type of physical euthanasia technique performed. Since most physical methods involve trauma, there is inherent risk for animals and humans, therefore extreme care and caution should be used. Methods not performed correctly can result in animal and personnel injuries. Inexperienced persons must be trained by experienced personnel and should practice on carcasses until they are proficient in performing the method properly and humanely.

Cervical Dislocation: Cervical dislocation is a technique that is rapidly accomplished and can induce rapid loss of consciousness without chemically contaminating tissue, but requires technical proficiency. Cervical dislocation is a humane technique for euthanasia of rodents weighing ≤200 g when performed correctly. In lieu of demonstrated technical competency, animals must be unconscious or anesthetized prior to cervical dislocation.

This technique can be used as a primary means of euthanasia only when scientifically justified by the PI, and approved by the IACUC. Those responsible for the use of this technique must ensure that personnel performing cervical dislocation techniques have been properly trained and consistently apply it humanely and effectively.

Decapitation: Decapitation is a technique that is rapidly accomplished and induces near instantaneous death without chemically contaminating tissues. Personnel performing this technique should recognize
the inherent danger of the guillotine or other sharp instruments and take adequate precautions to prevent personal injury. This method of euthanasia can only be used when its use is required by the experimental design and approved by the IACUC (except for animals younger than 10 days old). The equipment used to perform decapitation should be maintained in good working order and serviced on a regular basis to ensure sharpness of blades. The use of plastic cones to restrain animals appears to reduce distress from handling, minimizes the chance of injury to personnel, and improves positioning of the animal in the guillotine. Those responsible for the use of this technique must ensure that personnel who perform decapitation techniques have been properly trained to do so.

**Euthanasia in Anesthetized, Laparotomized (Non-Survival Surgery) Animals:**

While animals are fully anesthetized, they may be euthanized using one of the following acceptable methods of physical euthanasia:
- exsanguination - great vessels severed, cardiac perfusion, or removal of vital organs
- incision of the chest cavity or diaphragm to produce a pneumothorax (collapsed lung) and cessation of respiration
- decapitation
- cervical dislocation

**Unacceptable Methods of Euthanasia**

The following methods of euthanasia are expressly forbidden under any circumstance: air embolism, blow to the head/stunning, burning, chloral hydrate, chloroform, cyanide, decompression, drowning, formalin, household products and solvents such as acetone, quaternary compounds (including CCl₄), laxatives, clove oil, dimethyl-ketone, quaternary ammonium products (i.e., Roccal D Plus), antacids (and other commercial and household products), hypothermia, neuromuscular blocking agents, rapid freezing, and strychnine.

**Any exceptions to this policy must have IACUC and Veterinary approval**

**References:**


SECTION 3: Euthanasia of Rodent Fetuses and Neonates (Embryonic day 15 - postpartum day 9)

The 2013 AVMA Guidelines on Euthanasia provides limited recommendations for the euthanasia of prenatal or neonatal animals. Regarding prenatal and neonatal euthanasia, the 2013 Guidelines state: “When ovarian hysterectomies are performed, euthanasia of fetuses should be accomplished as soon as possible after removal from the dam. Neonatal animals appear to be resistant to hypoxia” (1). However, the Report of the ACLAM Task Force on Rodent Euthanasia provides greater detail for these young animals (2). In all cases, the person performing the euthanasia must be fully trained in the appropriate procedures.

Fetuses: At approximately 60% of the gestation period, the neural tube has developed into a functional brain and the likelihood that a fetus may perceive pain should be considered. Reflexive behavior in response to painful stimuli has been observed in fetuses and correlates with adult behaviors. However, fetal behavioral arousal and awareness may be suppressed by low arterial oxygen limiting higher cortical processing.

Mouse, Rat and Hamster fetuses up to 14 days (E14) and Guinea Pig fetuses up to 34 days (E34) gestation: Neural development at this stage is minimal and pain perception is considered unlikely. Euthanasia of the mother or removal of the fetus should ensure rapid death of the fetuses due to loss of blood supply and non-viability of fetuses at this stage of development. Therefore, it is unnecessary to remove fetuses for euthanasia after the dam is euthanized.

Mouse, Rat and Hamster fetuses 15 days gestation (E15) to birth and Guinea Pig fetuses 35 days gestation (E35) to birth: The neural development at this stage supports the likelihood that pain may be perceived. When fetuses are required for study, euthanasia of individual fetuses may be induced by the skillful injection of chemical anesthetics. Decapitation with surgical scissors or cervical dislocation are acceptable physical methods of euthanasia. Rapid freezing, without prior anesthesia, as a sole means of euthanasia is not considered to be humane. Animals should be anesthetized prior to freezing. When chemical fixation of the whole fetus is required, fetuses should be anesthetized prior to immersion in or perfusion with fixative solutions. Anesthesia may be induced by hypothermia of the fetus (3), or by injection of the fetus with a chemical anesthetic. The Veterinarian should be consulted for considerations of fetal sensitivity to specific anesthetic agents. Fetuses at this age are resistant to hypoxia and require extended exposure to inhalant anesthetics.

When fetuses are not required for study, the method chosen for euthanasia of a pregnant mother should ensure rapid cerebral anoxia to the fetus with minimal disturbance to the uterine milieu minimizing fetal arousal. Recommended methods for euthanasia of the mother are CO2 exposure followed by cervical dislocation. Death of the mother must be verified after euthanasia and prior to disposal. The Veterinarian should be consulted for considerations of other euthanasia agents.

Neonates: Maturation of nociceptors and the development of excitatory and inhibitory receptor systems occur during the period just prior to birth and into the second week of postnatal life (P13-16). Resistance to hypoxia at this age results in a prolonged time to unconsciousness when CO2 is used as a euthanasia agent and so this method is not permitted. Death must be verified after euthanasia and prior to disposal.
Mouse, Rat and Hamster up to 10 days (P10) of age: Acceptable methods for euthanasia include injection of chemical anesthetics (e.g., pentobarbital), decapitation, or cervical dislocation. The Veterinarian should be consulted for appropriate alternatives if needed.

Guinea Pig: Follow guidelines for adults (precocial young).

References -


SECTION 4: Effects of Various Methods of Euthanasia on Physiology

| Table 1: Biologic effects of decapitation (3, 5, 16, 49, 56, 60, 66) |
|-------------------------------------------------|--------------------------|
| **Effect**                                      | **Mechanism**            |
| Increase in plasma sodium                       | Hemolysis                |
| Increase in plasma potassium                    |                          |
| Increase in GABA concentrations (brain)         | Continued postmortem neurochemical alterations |
| Increase in Alanine (brain)                     |                          |
| Increase in plasma ascorbic acid (30-40% > resting state) |                          |
| Increase in blood catecholamine levels          |                          |
| Increased plasma calcium, magnesium             |                          |
| No change in vasoactive intestinal peptides (brain) |                          |
| No change in neuropeptide Y (brain)             |                          |
| Alteration in rat heart mitochondria function   |                          |
| Increase in serum corticosterone                | Stress stimulus → mobilization from tissues to blood; generalized metabolic response secondary to sympathoadrenal response; some handling related stimulation. |
|                                                  | Possible handling stress |

| Table 2: Effects of physical and pharmacological euthanasia methods |
|-------------------------------------------------|--------------------------|
| **Method**                                      | **Physiologic effect**   |
| Methoxyflurane and decapitation (10)            | Increase in prostacyclin (vasodilator that inhibits platelet aggregation) Vascular contractility suppressed Decreased vascular contractility |
| Ether and decapitation, or decapitation alone (50) | No statistical difference in prolactin levels or LH/FSH secretory properties of cultured anterior pituitary cells |
| Ether and decapitation (74)                     | No change in estrogen receptors/progesterone receptors in rat uteri |
| Ketamine and decapitation (50, 74)              | No change in estrogen receptors/progesterone receptors in rat uteri |
| Pentobarbital and decapitation (4)              | Increase in acetylcholine release in the brain |
| Halothane and decapitation (21)                 | Increase in plasma ascorbic acid Increase in plasma catecholamines |

| Table 2: Effects on reproductive hormones: The following combinations may be unsuitable for studies of serum androgens |
|-------------------------------------------------|--------------------------|
| Decapitation in combination with agents listed below (49, 71) | Male rats | Immature | Mature | Mechanism: direct effect on testes | Circulating Androstenedione |
|                                                  | LH | FSH | Prolactin | Testosterone | LH | FSH | Prolactin | Testosterone | Castrated | Intact |
| Xylazine                                         | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |
| Bicilin                                         | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |
| Thiopental                                       | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |
| Pentobarbital                                    | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |
| Ketamine                                         | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |
| Halothane                                        | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |
| Ether (tested on castrated rats)                 | -  | -   | -         | -         | -  | -   | -         | -         | -         | -     |

\[\text{↓ = decreased} \quad \text{↑ = increased} \quad \text{- = no change}\]
<table>
<thead>
<tr>
<th>Method of euthanasia</th>
<th>Effect</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injectable Pentobarbital&lt;sup&gt;1,2&lt;/sup&gt; (5, 53, 61)</td>
<td>Decreased muscular contractility in isolated muscle preps&lt;br&gt;Decreased O1 smooth muscle contractility when given orally or intravenously; not seen in intraperitoneal route&lt;br&gt;Intraperitoneal administration causes increased colonic contractility in response to acetylcholine&lt;br&gt;Decreased spontaneous and drug induced vascular smooth muscle contractility&lt;br&gt;Decreased catecholamine levels&lt;br&gt;Increased partial pressure of CO&lt;sub&gt;2&lt;/sub&gt; in arterial blood&lt;br&gt;Increased serum activity renin&lt;br&gt;Increased plasma aldosterone&lt;br&gt;Splenic enlargement&lt;br&gt;Increased plasma glucose and insulin&lt;br&gt;Increased liver glycogen&lt;br&gt;Decreased plasma triglycerides&lt;br&gt;Increased in plasma insulin</td>
<td>Decreased calcium transport&lt;br&gt;Increased CO&lt;sub&gt;2&lt;/sub&gt; in arterial blood may change blood pH, which then changes metabolic indices&lt;br&gt;Increased glucose production or decreased glucose clearance</td>
</tr>
<tr>
<td>Cervical dislocation/cervical fracture (32, 68, 72)</td>
<td>Decreased coronary flow; decreased contractile function in isolated perfused heart preparations</td>
<td>Possible decreased sensitivity of B-adrenergic receptors secondary to cervical fracture</td>
</tr>
<tr>
<td>Cervical dislocation and methoxyflurane (32)</td>
<td>Increased mitogen induced lymphocyte proliferation&lt;br&gt;Normal cytolytic T lymphocytes (CTL) response</td>
<td></td>
</tr>
</tbody>
</table>
### ADDITIONAL FACTORS THAT INFLUENCE THE OUTCOME OF EUTHANASIA: (6, 7, 18, 22, 38, 56)

- **Handling**: May cause sympathoadrenal discharge, which affects plasma glucose, progesterone plasma catecholamines. Habituating the animals to handling may mitigate this effect.
- **Environmental stimuli** (e.g., noise) can increase plasma corticosterone concentrations.
- **Sequence**: The order of euthanasia for rats housed in pairs produced significant differences in plasma tryptophan and unesterified fatty acids, plasma corticosterone, plasma protein lactate levels, substance P, cholecystokinin, somatostatin.

| 70% CO₂/30% O₂ vs 100% (Pre-charged) [52] | Decreased number of circulating CD3⁺ and CD8⁺ T cells Increase in CD19⁺ B cells in circulation |
| 70% CO₂/30% O₂ vs 100% (Not pre-charged) [52] | Increased number of circulating CD3⁺, CD4⁺, and CD8⁺ T cells |
| Isoflurane (8) | No change in liver glycogen |

**Table 4: Anesthetics – ketamine hydrochloride, pentobarbital, chloral hydrate, chloralose and halothane in combination**

| Fructose 2-6-biphosphate (35) | Significant increase in brain, heart, skeletal muscle concentrations |

**Table 5: Gross/histopathology changes (1, 24, 25, 33, 64)**

<table>
<thead>
<tr>
<th>Ether</th>
<th>Decapitation</th>
<th>CO₂¹</th>
<th>Methoxyflurane</th>
<th>Pentobarbital</th>
<th>Physical Methods (DC, CD)</th>
<th>Methods Listed in this Chart</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung: interstitial edema, marked alveolar emphysema</td>
<td>Lung: congestion, hemorrhage, blood in alveolar spaces</td>
<td>Lung: congestion, hemorrhage, emphysema, atelectasis. <strong>Cardiac muscle</strong>: variable degenerative changes (influenced by time of exposure to CO₂ causing acids, hypoxia). CO₂ + O₂ Lung: severe edema and hemorrhage, extravasation to alveoli. <strong>Cardiac muscle</strong>: variable degenerative changes (influenced by time of exposure to CO₂ causing acids, hypoxia). capillary bleeding causing marked extravasation of blood.</td>
<td>Lung: congestion <strong>Spleen</strong>: splenomegaly</td>
<td>Lung: emphysema congestion <strong>Spleen</strong>: emphysema, congestion <strong>GI serosa</strong>: emphysema, congestion <strong>Cardiac muscle</strong>: Acute degenerative lesions <strong>Kidney cortex</strong>: circulatory changes <strong>Other</strong>: Peritoneal congestion, sanguinous fluid in abdominal cavity</td>
<td>Lung: emphysema, bleeding <strong>Neck/Brain</strong>: local tissue trauma</td>
<td>No change in sperm motility</td>
</tr>
</tbody>
</table>

*NOTE: DC (decapitation), CD (cervical dislocation), CO₂ Intracardiac pentobarbital more suitable for histology of abdominal viscera*
References


Policy 5 – Body Weight Loss

Version 2.0
Approval Date: 1/22/07, 3/5/13

Purpose – The purpose of this policy is to provide guidelines to investigators as to what are acceptable and not acceptable losses in animal body weight and/or body condition.

Background –

“...animals should be closely monitored to ensure that food and fluid intake meets their nutritional needs...” – the Guide

Body Condition Scoring (BCS):

The scorer picks up the mouse at the base of the tail and passes a finger over the sacroiliac bones (dorsal pelvis). Body condition is typically scored on a scale of 1-5, as described below: (see Figure 1)

1. Muscle wasting is advanced, fat deposits are absent, and bones are very prominent.
2. Bones are prominent. This suggests the mouse is becoming thin and its health is declining. Further decline of condition would warrant euthanasia.
3. Bones are palpable but not prominent. This is the optimal condition.
4. The mouse is well fleshed and bones are barely felt.
5. The mouse is obese and bones cannot be felt.

Policy –

Indications for euthanasia:

1. Weight loss ≥15% within one week (7d) that cannot be corrected by fluid therapy and is due to loss of lean body mass
2. Overall weight loss ≥20% or BCS ≤ 1.5

Note: BCS should be used when body weight does accurately reflect the mouse’s condition (such as tumors, pregnancy, ascites, juvenile rodents ≤ 50 days).

Any exceptions to this policy must have IACUC and Veterinary approval
References -

Policy 6 – Cell Line Usage and Rodent-Derived Biological Products

Version 2.0
Approval Date: 1/22/07, 3/5/13

Purpose – The purpose of this policy is to provide investigators with information regarding the proper testing of certain products to be introduced into live rodents. This is part of the health surveillance program at RWJMS, created to prevent the introduction of infectious diseases into animal use facilities.

Background - Cell lines, tissues and body fluids that have been derived from or passed through rodents can harbor infectious agents and contaminate in-house rodent colonies causing large scale, costly, deleterious effects to the animal research program and human health (1,2,3). Transplantable tumors, hybridomas, cell lines, blood products, and other biologic materials can also be sources of both murine and human viruses that can contaminate rodents or pose serious risks to laboratory personnel (4); rapid and effective assays are available to monitor microbiologic contamination and should be considered before introducing such material into animals (5,6).

Policy –

The following agents must be tested before introducing into the animals:

1. All cell lines of rodent origin obtained from sources that have not been tested for and documented free of murine pathogens that are being administered to rodents (mouse, rat, hamster) at RWJMS.

2. Any cell lines passed through rodents, including human cell lines.

3. Rodent body fluids (blood and serum), cells, and tissues obtained from sources that have not been tested for and documented free of murine pathogens and intended for use in rodents at RWJMS. This includes rodent sera for use in cell cultures.

Required testing:

IDEXX RADIL Lab Animal and Biological Materials Diagnostic Testing
email: idexx-radil@idexx.com
Phone: 800-669-0825; 573-499-5700
Fax: 573-499-5701

email: preclinicalresearch@idexx.com
Phone: 800-444-4210 option 5
PCR - Infectious Microbe PCR Amplification Test (IMPACT):

Mice - IMPACT Profile I (20 agent test)

Rats - IMPACT Profile V (16 agent test)

Other laboratories can be used, but must be pre-approved by the TRC Director.

Reporting - Information on the proposed use of rodent cell lines/biologicals must be provided on the IACUC application. Copies of test results must be submitted to the TRC Director for review and approval, prior to the actual use of rodent cell lines. Addition of new cell line(s) for ongoing approved projects also require testing, review, and approval prior to use.

Any exceptions to this policy must have IACUC approval

References -


Policy 7 – Experimental Allergic Encephalomyelitis (EAE) in Mice

Version 2.0
Approval Date: 1/22/07, 3/5/13

**Purpose** - The purpose of this policy is to describe the specialized care of EAE animals, in order to ensure their humane care and treatment.

**Background** - Experimental allergic encephalomyelitis (EAE) is an animal model for central nervous system autoimmune disease; it is widely used as a human Multiple Sclerosis (MS) model. Although clinical signs vary according to species and strain, they typically include visual, sensory, and motor deficits. This generally manifests as an ascending paralysis graded on a five-point scale ranging from the loss of tail tone in an otherwise normal animal (1) to one in a moribund condition (5). The course may vary from one or more episodes with short periods of remission of clinical signs, to a progressive, chronic state.

**Policy**

**Care of EAE mice:**

1. Every animal to be injected with any substance to elicit EAE should be identified with a cage card with the letters “EAE”.
2. At the time of inoculation, post-procedural cage card (blue card) specifying “EAE mice” and date of inoculation should be placed in every applicable cage.
3. Once animals develop clinical signs, animals should be monitored at least daily, including weekends and holidays.
4. Food should be placed on the floor of the cage and a water bottle with a long sipper tube should be used. An alternative source of water should be provided (i.e. Napa nectar).
5. Monitoring will include the graded score of EAE development (see Table 1), hydration status, body condition score, general condition, and activity level of the animal(s). All observations and treatments must be recorded on the blue post-procedural cage card, dated and initialized.
6. All paralyzed mice should be monitored for skin irritation associated with urine scalding and, if male, observed for penile irritation secondary to flaccid paralysis.
7. Paralyzed animals should be removed from the cage if healthy mice compromise their health. Healthy animals may walk on paralyzed animals, causing discomfort and/or injuries, and may eat food intended for paralyzed animals.

**Humane endpoints (euthanasia indicated):**

- EAE grade 4 and 5 (see EAE clinical signs and interventions table for exceptions)
- BCS ≤1.5 or body weight loss ≥20%
- Veterinarian recommendation
| N/A   | Normal                                      | 1. Baseline weight  
|       |                                             | 2. Write "EAE" on record cage card  
|       |                                             | 3. Write immunization date  |
| early | Tail tone: Decreased tail tone              | 1. Start record keeping (activity,  
|       |                                             | EAE stage#)  
|       |                                             | 2. Monitor daily  |
| early | Hind limb Paresis: Weakness in hind limbs, Animals have difficulty to move, appear ataxic or "clumsy" | 1. Monitor BW 1X a week and record  
|       |                                             | 2. Use long sipper tubes, soft food  
|       |                                             | Or gel diets  
|       |                                             | 3. Provide moistened food pellets on cage floor  |
| middle| Hind limb paralysis: Inability to move one or both hind limbs  
|       | Urinary Incontinence: Urine is leaking, urine scald around prepuce or vulva  
|       | Dehydration: Decreased skin turgor  
|       | Oral/lingual paralysis: Inability to swallow  | 1. Follow stage 2  
|       |                                             | 2. Palpate bladder 3X/week  
|       |                                             | 3. Monitor for skin lesions, urine scald, penile prolapse  
|       |                                             | 4. Use SC fluid for dehydration  
|       |                                             | 5. Use softer bedding materials (alpha dry)  
|       |                                             | 6. Provide food via oral gavage if animal unable to swallow  |
| Middle to late | Weakness of fore limbs with paraparesis or quadriparesis  
|       | Atonic bladder: Enlarged, unable to urinate  
|       | Dehydration: Decreased skin turgor  
|       | Oral/Lingual paralysis: Inability to swallow  | 1. Follow stage 3  
|       |                                             | 2. For atonic bladder, express bladder at least 2X/day.  |
| Late End stage | Quadriplegia: Paralysis of all four limbs  
|       | Atonic bladder: Enlarged, unable to urinate  
|       | Dehydration: Decreased skin turgor  
|       | Oral/lingual paralysis: Inability to swallow  
|       | Dyspnea: Difficulty or abnormal breathing  
|       | Moribund: Animal is not moving, recumbent  | 1. Follow stage 4  
|       |                                             | 2. Animals must be euthanized unless exempted by the IACUC  
|       |                                             | 3. All moribund, body weight loss<20%, dyspenic animals must be euthanized  |
Table 1- Clinical grading of EAE mice

<table>
<thead>
<tr>
<th>EAE GRADE</th>
<th>CLINICAL SIGNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Normal mouse; no overt signs of disease</td>
</tr>
<tr>
<td>1</td>
<td>Decreased tail tone or weak tail only</td>
</tr>
<tr>
<td>2</td>
<td>Hind limb weakness (paraparesis)</td>
</tr>
<tr>
<td>3</td>
<td>Hind limb paralysis (paraplegia) and/or urinary incontinence</td>
</tr>
<tr>
<td>4</td>
<td>Weakness of fore limbs with paraparesis or paraplegia (quadriplegia) and/or atonic bladder</td>
</tr>
<tr>
<td>5</td>
<td>Paralysis of all limbs (quadriplegia), moribund state; death by EAE</td>
</tr>
</tbody>
</table>

Any exceptions to this policy must have IACUC approval

References -


Policy 8 – Tissue Collection for Genotyping of Mice and Rats

Version 2.0
Approval Date: 1/22/07, 3/5/13

Purpose - The tissue obtained from the tail of a rodent can be used for genetic analysis. Analysis by Polymerase Chain Reaction (PCR) requires the least amount of DNA. Alternatively, DNA for PCR analysis can also be obtained from ear punches, hair/fecal samples, and oral/rectal swabs (1-11). Investigators are encouraged to maintain animals in a homozygous state whenever possible in order to minimize (or eliminate) the need for tail biopsy. Furthermore, using PCR vs. Southern blot for genotyping, when possible, reduces the tissue requirement and permits using ear punches and saliva for genotyping.

Background - The tail of a mouse contains a variety of tissues, including bone, cartilage, blood vessels and nerves. In young mice (<17 days) the tissue near the tip of the tail is soft and the bones have not completely mineralized. Therefore, removing of the tail tip of a young mouse probably amounts to no more than momentary pain for the animal (13). As the animal ages, however, mineralization of the bone and increased vascularity progresses in this region, requiring anesthesia to perform the same procedure in the same location. Therefore, mice and rats should ideally be 17-21 days old for tail biopsy, in order to yield the most tissue with the least amount of pain. In addition, prompt analysis of tail tissue allows mice to be identified prior to weaning, which can facilitate more efficient use of cage space.

Policy –

- mice and rats ≤21 days - no anesthetic or analgesia is required for ≤5mm
- mice and rats 22-28 days - under general or local anesthesia; no analgesia required
- mice and rats ≥26 days - under general anesthesia with analgesia (buprenorphine or carprofen)

Table 1 - Summary of the tail clipping requirements

<table>
<thead>
<tr>
<th>age</th>
<th>anesthetic</th>
<th>analgesia</th>
<th>hemostasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤21 days biopsy ≤5mm</td>
<td>no</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>22-28 days biopsy ≤5mm</td>
<td>yes</td>
<td>no</td>
<td>yes</td>
</tr>
<tr>
<td>≥29 days biopsy ≤5mm</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>any age, biopsy ≥6 mm</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
Procedure -

- After administering anesthetic (if required), manually restrain mouse or rat between thumb and forefinger, disinfect tail with 70% isopropyl alcohol; using a sterile scalpel, razor blade, or scissors cleanly excise distal segment of tail (≤5 mm). If small amounts of DNA are required, investigators should consider harvesting 2mm of tail.
- If the DNA analysis is to be performed by PCR, great care should be taken to remove all tissue from the scissors or scalpel after each animal and the scalpel or scissors should be disinfected between animals. If a scalpel is used, disinfect the work/cutting surface on which the tail is placed between animals.
- The investigator must monitor animal(s) to assure hemostasis after animal(s) are returned to the cage. The presence of blood in the cage may cause aggression between cage mates. Apply digital pressure, clotisol, or other means of hemostasis to stop bleeding.

Anesthetic - General anesthesia using 4% isoflurane; injectables are also permitted (see Policy 10). For local anesthetic, soak tail in 0.75% bupivacaine solution for 30s before harvesting.

Analgesia - pre-emptive analgesia using buprenorphine (0.1mg/kg mice, 0.05mg/kg rat) or carprofen (4 mg/kg), subcutaneously or PO; additional dosing may be required if animals show continued signs of pain.

Any exceptions to this policy must have IACUC approval

References -


**Purpose** – The purpose of this policy is to provide investigators directions for the proper techniques associated with toe clipping, an acceptable, permanent form of animal identification.

**Background** – Toe clipping is a method that involves a numerical scheme in which the first bone of certain toes is removed with a sharp instrument for identification of small rodents.

“As a method of identification of small rodents, toe clipping should be used only when no other individual identification method is feasible. It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age (6,7), especially if toe clipping and genotyping can be combined. Under all circumstances aseptic practices should be followed. Use of anesthesia or analgesia should be commensurate with the age of the animals (8).” – the *Guide* (1)

**Policy** –

Toe clipping will be considered under the following conditions:

1. **Justification:** A written explanation to the IACUC of why it is necessary, including why alternate methods are unsatisfactory.

2. **Animal age:** Toe clipping should only be performed when mice are between 1-12 days; the preferred age is 7 days old. (6)

3. **Number of toes:** Limited to a maximum of four toes and no more than one per foot. Do not cut the hallux (“dew-claw” or “little toe” of the forepaw) as this may decrease the rodent's grasping ability. Also, confirm bleeding has stopped prior to returning animal(s) to cage(s).

**Procedure:**

1. No anesthetic is required for animals age ≤7 days old; animals between 8-12 days old require use of general anesthesia.
2. Use sharp scissors or a blade sanitized with 70% ethanol or antiseptic solution (Clidox, povidone iodine, chlorhexidine, etc.)
3. If bleeding is observed, apply gauze with gentle pressure or Clotisol (anticoagulant powder) to the affected region for hemostasis.

Any exceptions to this policy must have IACUC approval
References -

1) The Guide for the Care and Use of Laboratory Animals, 8th ed, NRC Press, page 75.

2) NIH Guidelines for Toe Clipping of Rodents  

3) Yale University, IACUC Policy, revised 6-2006:  
   http://iacuc.yale.edu/policies/ToeClippingGuidelines.pdf

4) Dartmouth University IACUC policy, 2003  
   http://dms.dartmouth.edu/arc/iacuc/policies.shtml

5) Johns Hopkins University IACUC policy, 2005  
   http://www.jhu.edu/animalcare/committee_toe_clipping.html

6) Schaefer DC, Asner IN, Seifert B, Burki K, and Cinelli P. 2010. Analysis of physiological and  
   behavioral parameters in mice after toe clipping as newborns, Laboratory Animals 44: 7-13.


   collection in laboratory mice (Mus musculus): vertebral ossification, DNA quantity, and acute  
Purpose – The purpose of this policy is to provide investigators with guidelines regarding acceptable and unacceptable methods pertaining to rodent surgical technique.

Background – Both the Animal Welfare Act (AWA) and the NIH Guide for the Care and Use of Laboratory Animals contain standards for surgical procedures and surgical facilities.

“Successful surgical outcomes require appropriate attention to pre-surgical planning, personnel training, anesthesia, aseptic and surgical technique, assessment of animal well-being, appropriate use of analgesics, and animal physiological status during all phases of a protocol involving surgery and postoperative care” – the Guide

“Researchers conducting surgical procedures must have appropriate training to ensure that good surgical technique is practiced – that is asepsis, gentle tissue handling, minimal dissection of tissue, appropriate use of instruments, effective hemostasis, and correct use of suture materials and patterns” – the Guide

"All survival surgery will be performed using surgical gloves, masks, sterile instruments and aseptic techniques.... Non-major surgery and all surgery on rodents do not require a dedicated facility, but must be performed using aseptic technique." - Animal Welfare Act [9 CFR (code of Federal Regulations), Part 2, 2.31 (d)[1](ix)]

Policy –

Section I – Summary table for rodent survival surgery

Section II – Training

Section III – Types of surgery (survival (major, minor, multiple survival surgeries), non-survival)

Section IV – Surgery

- Pre-operative period (facility, instruments, animal(s), surgeon)
- Peri-operative period (animal monitoring, closing, analgesics)
- Post-operative period (recovery, animal monitoring, documentation, complications)

Section V – Assessment of post-operative pain

Section VI – Use of hypothermia as anesthesia in neonates

Section VII – Reference tables
Section I - Summary table of rodent survival surgery

<table>
<thead>
<tr>
<th></th>
<th>Training</th>
<th>All personnel performing surgery must have skills with performing surgery and anesthesia and must be evaluated successfully by the veterinary staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Aseptic methods</td>
<td>Clean lab coat, sterile instruments, surgical gloves, mask (except embryo transfer surgery), head cover, and aseptic procedures</td>
</tr>
<tr>
<td>3</td>
<td>Animal preparation</td>
<td>Shaving fur, disinfecting skin, eye lubrication, pre-emptive analgesia</td>
</tr>
<tr>
<td>4</td>
<td>Peri-op care</td>
<td>Monitoring depth of anesthesia, keeping animals warm</td>
</tr>
<tr>
<td>5</td>
<td>Post-op care</td>
<td>SC saline injection, keeping animal warm, monitoring animals until anesthetic recovery, administering additional analgesia, monitoring for post-op complications</td>
</tr>
<tr>
<td>6</td>
<td>Analgesics</td>
<td>Major surgeries must receive 24 hours of pain medications, minor surgery 1 dose (or as stated in the IACUC protocol or recommended by the veterinary staff), additional analgesia may be needed if animal show signs of pain</td>
</tr>
<tr>
<td>7</td>
<td>Local anesthetic</td>
<td>Required for all incisions</td>
</tr>
<tr>
<td>8</td>
<td>Blue card</td>
<td>Required for post-op records</td>
</tr>
<tr>
<td>9</td>
<td>Suture removal</td>
<td>Required removal 10-14 days post-op</td>
</tr>
<tr>
<td>10</td>
<td>Euthanasia</td>
<td>Animal with uncorrectable post-op complications (e.g., persistent infection, organ failure) must be euthanized.</td>
</tr>
</tbody>
</table>

Section II - Training

Professional and technical personnel along with students who perform anesthesia and surgery should be appropriately trained to accomplish these tasks in a humane and scientifically acceptable manner. The investigator is responsible for assuring that research personnel receive appropriate training. The TRC staff is available to provide assistance with, or training in, aseptic technique and the proper administration of anesthesia, analgesia, and euthanasia.

Section III – Types of Surgery

- Survival surgery
  - Major survival surgery
  - Minor survival surgery
- Non-survival surgery
- Multiple, major survival surgery
**Major surgery** - Major survival surgery includes invasion of the abdominal or thoracic cavities. Procedures involving penetration of the cranial cavity can be either considered major or minor surgery, depending on the specific procedure. In addition, any procedure that might leave the rodent with a permanent handicap (whether physical or physiological) is also considered major surgery (ie, amputation of a limb, joint replacement). All major surgeries require administration of analgesics preoperatively and at least 24hrs postoperatively, unless the avoidance is scientifically justified and approved by the IACUC.

**Minor surgery** - Minor survival surgery does not expose a body cavity and causes little or no physical impairment (the *Guide*, p117). Examples of minor survival surgeries include placement of subcutaneous implants, peripheral vessel cannulation, and percutaneous biopsy. Most minor procedures require at least one pre-operative dose of analgesic.

**Subcutaneous implants** - The number and size of subcutaneous implants should be the lowest number and smallest size as possible. Subcutaneous implants must not impede normal mobility and physiologic function (ie, eating, defecation, urination and respiration) in the animal. Implants are sometimes delivered through a large-bore needle called a trocar. Because of the large diameter of a trocar (≤16ga), more than momentary pain is associated with their use. Therefore, all procedures involving trocars are considered minor survival surgery by the IACUC. Animals MUST be under general anesthesia or have a local anesthetic agent applied at the trocar site for these procedures. Trocars will cause more damage to the skin compared to smaller gauge hypodermic needles, and skin closure (suture, staples, wound clips, surgical glue) may be needed post-injection. Additionally, animals should be provided with analgesia for at least 12hrs postoperatively.

**Non-survival surgery** - In non-survival surgery, the animal is euthanized before recovery from anesthesia. It is not necessary to follow all techniques outlined in this policy for non-survival surgery, but, at a minimum, the surgical site should be clipped, the surgeon should wear gloves, and the instruments and surrounding area should be clean. For non-survival procedures of extended duration (>2hrs), attention to aseptic technique may be more important in order to ensure stability of the model and a successful outcome.

**Multiple survival surgeries (MSS)** – MSS involves successive surgical procedures in which the animal is repeatedly anesthetized (not multiple procedures performed during one anesthetic period). All efforts should be made to avoid multiple survival surgery in animal studies, especially with major surgeries. However, there are instances when investigators have a scientific need for the performance of multiple survival surgical procedures. Such procedures must be described in the protocol, scientifically justified, and approved by the IACUC.

**Section IV – Surgery**

- **Pre-operative period**

  **Surgical facility** - A separate facility for rodent surgery is not required. The surgical area must be clean, uncluttered, and free from overhanging objects (with the exception of light sources). The area
should be away from major traffic areas, doors and windows. The designated area should not be used for other activities during the surgical procedures and must be disinfected prior to the surgical session. Devices or equipment (ie animal restraining devices, monitoring equipment, stereotaxic devices, etc.) must also be disinfected in order to reduce or potentially eliminate infectious organisms (Table 1).

**Preparation of surgical instruments** - Instruments, implantable devices (catheters, osmotic pumps, telemetry) and suture material must be sterilized by using the methods listed in Table 3 (unless pre-sterilized by the manufacturer). The appropriate selection of suture material is essential; characteristics and uses of different materials are listed in Table 5.

**Animal preparation**
- Fur must be removed from the surgical site; either by clipping, plucking or using a depilatory in an area separate from where the surgery is to be conducted. An area approximately 15% larger than the area of the incision should be prepared.
- Clean and aseptically prepare the surgical site by using an appropriate scrubbing technique (e.g. scrubbing in gradually enlarging circular pattern from the interior of the shaved area to the exterior) and an effective disinfectant (e.g. alternating Betadine or Nolvasan™ and alcohol scrubs three times, see Table 2).
- Minimize soaking the body of the rodent; this may lead to irreversible hypothermia and death.
- The surgical area should be draped using either standard draping material, autoclaved paper towels or Steri-Drapes™.
- Animals must be kept warm by placing on a water re-circulating heating blanket or pads during surgery to prevent hypothermia.
- An ophthalmic ointment be placed in the anesthetized animals’ eyes to prevent drying of the cornea
- Withholding of food is not necessary in rodents unless specifically mandated by the protocol or surgical procedure.

**Surgeon preparation**
- The surgeon must wear a clean lab coat, head cover, mask (except embryo transfer surgery, because necessity of mouth pipetting) and sterile gloves.
- A sterile surgical gown is recommended for major or prolonged surgeries.
- The surgeon must scrub their hands before putting gloves on.

**Operative period**
- The animal must be maintained in a surgical plane of anesthesia throughout the procedure. Check pedal or tail pinch reflexes every 5 minutes. Pedal reflexes or tail reflexes are checked by gently pinching the animal's foot and determining whether the animal pulls or moves its foot or tail back. The movement of vibrassea also indicated low anesthetic depth. If the animal has an elevated respiration rate or positive pedal/tail reflex, supplement the anesthesia with one-half the initial dose (anesthetic drug minus tranquilizer) or increase the inhalant anesthetic dose by 0.5% increments; monitor the dosages carefully to avoid overdosing.
- It is required to infiltrate the surgical site with local anesthetic; bupivacaine (Marcaine®) is a long-acting local anesthetic. Infiltration of the surgical site provides local anesthesia for 8-12 hours post-operatively. [0.25% Marcaine, dilute 1:10 in sterile water, saline or PBS, administer total volume of 0.1 ml (mouse) or 1.0 ml (rat)]
- Begin surgery with sterile instruments (Table 3) and handle them aseptically. Instruments and gloves may be used for a series of similar surgeries provided they are remain clean and are disinfected (in 70% alcohol) between animals.
- Monitor and/or maintain the animal's vital signs:
  - Mucous membrane color - mucous membranes should remain pink
  - Breathing pattern - breathing should be regular and within normal range
- The exteriorizing of organs should be avoided if possible, but if required, should be placed on sterile drapes.
- Close surgical incisions using appropriate techniques and materials (Table 5).

Requirements for analgesics -
- Analgesics must be given pre-emptively, ie before incisions are made so plasma drug levels have reached the effective concentration. Analgesics are usually 30-60 minutes before surgery, then provided as needed (prn) depending on the procedure and the behavior of the animal(s).
- Animals must receive post-op analgesic coverage for the first 24 hours after all major surgeries. Minor surgeries may require at least one dose of a long lasting analgesic pre-operatively. Analgesics should be continued if signs of pain observed or as prescribed by the veterinarian (See Table 6); omission of analgesics requires prior IACUC approval.

Post-operative period
- Administer warm (25°C) sterile lactated ringers or 0.9% saline solution, 0.5-1 ml (mice) or 3-5ml (rats) subcutaneously (can also be given intraperitoneally but not recommended). If animal is unable to eat and drink the daily maintenance dose of fluids is 100 ml/kg/day for rodents. Fluids should be administered every 8 hours until animal is able to eat and drink in its own.
- Move the animal to a warm, dry area. The cage should be warmed to no greater than 25°C (85°F). To prevent hyperthermia, animals must be provided a means to escape the heat source once they are awake.
- Monitor animal(s) regularly (at least every 15 minutes if using injectable anesthetic or continuously for inhalation anesthetic) until animal is fully ambulatory. Return the animal to its regular housing only after it has fully recovered from anesthesia.
- Animal(s) must be monitored at least twice daily until there are no signs of pain or infection. Provide analgesics until no signs of pain are present.
- Monitor incision(s) for swelling, exudate, pain or dehiscence
- Monitor catheters and any other attached devices
- Monitor for procedure-related complications such as organ failure, thrombosis, and ischemia
- Remove skin closures 10 to 14 days post-operatively
- Use BLUE cards - Maintain records of surgeries and post-operative care. This includes procedure type, date of procedure, date and time of monitoring, all medications administered (dose and route), general animal appearance (signs of pain, dehydration, food and water intake).
- Rodents tend to cannibalize nonresponsive cage mates. Even if all rodents in a cage were anesthetized, some will be slower to recover than others, and may be injured by more alert animals. Therefore, it is best to recover rodents in separate cages until they are fully ambulatory.
- If the animal appears ill or the surgical wound appears abnormal, contact the TRC Veterinary staff immediately.

Section V - Clinical assessment of post-procedural pain (11)

<table>
<thead>
<tr>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Reduced grooming</td>
<td>- Reduced level of spontaneous activity</td>
</tr>
<tr>
<td>- Reduced level of spontaneous activity</td>
<td>- Increased back arching, horizontal stretching, abdominal writhing, falling/staggering, poor gait and twitching</td>
</tr>
<tr>
<td>- Piloerection</td>
<td>- Decreased grooming</td>
</tr>
<tr>
<td>- Hunched posture</td>
<td>- Porphyrin secretions (ocular/nares)</td>
</tr>
<tr>
<td>- Squinting</td>
<td>- Squinting</td>
</tr>
<tr>
<td>- Pale eyes (if albino)</td>
<td>- Pale eyes (if albino)</td>
</tr>
<tr>
<td>- Increased aggressiveness when handled</td>
<td>- Piloerection</td>
</tr>
<tr>
<td>- Distance themselves from cage mates</td>
<td>- Reduced food and water intake increased aggressiveness when handled</td>
</tr>
<tr>
<td>- Reduced food/water intake</td>
<td></td>
</tr>
</tbody>
</table>

Section VI - Use of hypothermia as anesthesia in neonates

The gradual cooling of fetuses and altricial neonates for anesthesia is acceptable with conditions. As cold surfaces can cause tissue damage (and presumably pain), animals should not come into direct contact with the ice / precooled surfaces. Hypothermia for anesthesia is not recommended after ~7 days of age.

Section VII – Reference tables

**Table 1. RECOMMENDED HARD SURFACE DISINFECTANTS** (e.g., table tops, equipment; always follow manufacturer's instructions)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol</td>
<td>Contact time required is 15 minutes, contaminated surfaces take longer to disinfect, remove gross contamination before using, inexpensive</td>
</tr>
<tr>
<td></td>
<td>85% isopropyl alcohol</td>
<td></td>
</tr>
<tr>
<td>Quaternary Ammonium</td>
<td>Roccal®, Cetylcide®</td>
<td>Rapidly inactivated by organic matter, compounds may support growth of gram-negative bacteria</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox® 10% solution)</td>
<td>Corrosive, presence of organic matter reduces activity, chlorine dioxide must be fresh (&lt;7 days), kills vegetative organisms within 10 minutes of contact</td>
</tr>
<tr>
<td></td>
<td>Chlorine dioxide, MB-10®</td>
<td></td>
</tr>
<tr>
<td>Aldehydes</td>
<td>Glutaraldehyde (Cidex®, Cide Wipes®)</td>
<td>Rapidly disinfects surfaces, toxic, exposure limits have been set by OSHA</td>
</tr>
<tr>
<td>-----------</td>
<td>-------------------------------------</td>
<td>---------------------------------------------------------------------</td>
</tr>
<tr>
<td>Phenolics</td>
<td>Lysol®, TBQ®</td>
<td>Less affected by organic material than other disinfectants</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity, rapidly bactericidal and persistent, effective against many viruses</td>
</tr>
</tbody>
</table>

Table 2. SKIN DISINFECTANTS

Alternating disinfectants is more effective than using a single agent. For instance, an iodophore scrub can be alternated 3 times with an alcohol, followed by a final soaking with a disinfectant solution. Alcohol, by itself, is not an adequate skin disinfectant. The evaporation of alcohol or alcohol-based products (e.g., Alcar, etc.) can induce hypothermia in small animals.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodophors</td>
<td>Betadine®, Prepodyne®, Wescodyne®</td>
<td>Reduced activity in presence of organic matter, wide range of microbicidal action; works best at pH 6-7</td>
</tr>
<tr>
<td>Cholorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity, rapidly bactericidal and persistent, effective against many viruses, excellent for use on skin</td>
</tr>
</tbody>
</table>

Table 3. RECOMMENDED INSTRUMENT STERILANTS (Always follow manufacturer's instructions)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical: Steam</td>
<td>Autoclave</td>
<td>Effectiveness dependent upon temperature, pressure, and time (ie 121°C for 15min vs. 131°C for 3min)</td>
</tr>
<tr>
<td>Sterilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry Heat</td>
<td>Hot bead sterilizer</td>
<td>Fast, instruments must be cooled before contacting tissue</td>
</tr>
<tr>
<td></td>
<td>Dry chamber</td>
<td></td>
</tr>
<tr>
<td>Ionizing radiation</td>
<td>Gamma radiation</td>
<td>Requires special equipment</td>
</tr>
<tr>
<td>Chemical: Gas</td>
<td>Ethylene oxide</td>
<td>Requires 30% or greater relative humidity for effectiveness against spores, gas is irritating to tissue, all materials require safe airing time before use</td>
</tr>
<tr>
<td>Sterilization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorine¹</td>
<td>Chlorine dioxide</td>
<td>Minimum of 6 hours required for sterilization, presence of organic matter reduces activity, must be freshly made (&lt;7d)</td>
</tr>
<tr>
<td>Aldehydes¹</td>
<td>Formaldehyde (6% sol)</td>
<td>For all aldehydes: many hours required for sterilization, corrosive and irritating, consult safety representative on proper use, glutaraldehyde is less irritating and less corrosive than formaldehyde</td>
</tr>
</tbody>
</table>

¹: Formaldehyde and glutaraldehyde are autoclave unstable.
Table 4. RECOMMENDED INSTRUMENT DISINFECTANTS (Always follow manufacturer's instructions)

<table>
<thead>
<tr>
<th>Agent</th>
<th>Examples</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohols</td>
<td>70% ethyl alcohol, 85% isopropyl alcohol</td>
<td>Contact time required is 15 minutes, watch for evaporation; contaminated surfaces take longer to disinfect, remove gross contamination before using, inexpensive</td>
</tr>
<tr>
<td>Chlorine</td>
<td>Sodium hypochlorite (Clorox® 10% solution), Chlorine dioxide (Clidox®, Alcide®)</td>
<td>Corrosive, presence of organic matter reduces activity, chlorine dioxide must be fresh (&lt;7 days old), kills vegetative organisms within 3 min</td>
</tr>
<tr>
<td>Chlorhexidine</td>
<td>Nolvasan®, Hibiclens®</td>
<td>Presence of blood does not interfere with activity, rapidly bactericidal and persistent, effective against many viruses</td>
</tr>
</tbody>
</table>

Instruments must be rinsed thoroughly with sterile water or saline to remove chemical sterilants before being used.

Table 5. SUTURE SELECTION

<table>
<thead>
<tr>
<th>Suture</th>
<th>Characteristics and Frequent Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vicryl®, Dexon®</td>
<td>Absorbable (60-90d), ligate or suture tissues where an absorbable suture is desirable</td>
</tr>
<tr>
<td>PDS® or Maxon®</td>
<td>Absorbable (6 months), ligate or suture tissues especially where an absorbable suture and extended wound support is desirable</td>
</tr>
<tr>
<td>Prolene®</td>
<td>Non-absorbable, inert</td>
</tr>
<tr>
<td>Nylon</td>
<td>Non-absorbable, inert, general closure</td>
</tr>
<tr>
<td>Silk</td>
<td>Non-absorbable, excellent handling, preferred for cardiovascular procedures, tissue reactive and may wick microorganisms into the wound, <strong>not acceptable for suturing skin</strong></td>
</tr>
<tr>
<td>Chromic Gut</td>
<td>Absorbable, versatile</td>
</tr>
<tr>
<td>Stainless Steel Wound Clips, Staples</td>
<td>Non-absorbable, requires instrument(s) for skin removal</td>
</tr>
</tbody>
</table>

Table 6. LONG ACTING ANALGESIC DOSE RATES

<table>
<thead>
<tr>
<th>Species</th>
<th>Opioid analgesics (buprenorphine/buprenex)</th>
<th>NSAID (carprofen)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>0.1 mg/kg SC, q8-12h</td>
<td>5 mg/kg SC, q24h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>one Rimadyl (2mg) tablet (Bio-Serv) for 25g mouse, replaces standard diet</td>
</tr>
<tr>
<td>Rat</td>
<td>0.01-0.05 mg/kg SC q8-12h</td>
<td>5 mg/kg SC, q24h</td>
</tr>
<tr>
<td></td>
<td></td>
<td>three Rimadyl (2mg) tablets (Bio-Serv) for 300-400g rat, replaces standard diet</td>
</tr>
</tbody>
</table>
Table 7. MOUSE INJECTABLE ANESTHETIC DOSE RATES

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine/xylazine</td>
<td>100 mg/kg / 10 mg/kg IP</td>
</tr>
<tr>
<td>Ketamine/acepromazine</td>
<td>100 mg/kg / 5 mg/kg IP</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>4% induction, then titrate to effect</td>
</tr>
</tbody>
</table>

Table 8. RAT INJECTABLE ANESTHETIC DOSE RATES

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketamine/xylazine</td>
<td>75(-100) mg/kg / 10 mg/kg IP</td>
</tr>
<tr>
<td>Ketamine/acepromazine</td>
<td>75mg/kg / 2.5 mg/kg IP</td>
</tr>
<tr>
<td>Isoflurane</td>
<td>4% induction, then titrate to effect</td>
</tr>
</tbody>
</table>

References -

2. The Guide for the Care and Use of Laboratory Animals, 8th ed, NRC press. [http://www.nap.edu/readingroom/books/labrats/chaps.html#sur](http://www.nap.edu/readingroom/books/labrats/chaps.html#sur)
7. NIH, Training in Survival Rodent Surgery, 2005 (CD-ROM). For a free copy send email to: [rodentcd@od.nih.gov](mailto:rodentcd@od.nih.gov)
9. NIH, Training in Basic Biomethodology for Laboratory Mice. 2005 (CD-ROM). For a free copy send email to: [rodent-cd@mail.nih.gov](mailto:rodent-cd@mail.nih.gov)
Purpose – The purpose of this policy is to instruct investigators as to the proper use, storage, and documentation of drugs (especially concerning controlled substances and non-pharmaceutical grade compounds) and the acceptable use of expired medical materials (syringes, suture, needles, etc.).

Background –

The use of expired pharmaceuticals, biologics, and supplies is not consistent with acceptable veterinary practice or adequate veterinary care (1,2,3).

Definitions:

Expiration - The month after the date indicated on the container

Non-drug medical materials - Includes things like sutures, wound clips, catheters, needles, and syringes

Non-survival procedure (aka acute procedure, terminal procedure) – A procedure in which the animal is euthanatized before anesthetic recovery (3)

Survival procedure - A procedure from which the animal recovers (from anesthesia)

Pharmaceutical grade – Compounds or agents intended for use in human or veterinary medicine; meet the highest standards for purity and bioavailability

Chemical grade – Compounds or agents which may be chemically identical to their pharmaceutical grade counterparts, but do not conform to recognized standards for purity and bioavailability

Controlled drugs (4) - Controlled substances are drugs (or other substances) regulated under the Controlled Substances Act (CSA, enforced through the DEA and FDA). The scheduling of a particular drug (I-IV) depends on medical use, potential for abuse, and safety. Controlled substance drugs are labeled with a ‘C’ and the corresponding schedule number. Examples of controlled drugs that are commonly used in research animals include ketamine (III), buprenorphine (III), sodium pentobarbital, and Euthasol (III).
Policy –

Expired drugs -

- Include name, concentration, and expiration date on each drug container aliquoted from stock solutions
- All dilutions and mixtures of drugs are to be discarded after one month from the date of preparation, unless a longer/shorter dilution shelf life is specified by the manufacturer
- All expired drugs should be discarded as soon as possible through the appropriate channels (especially important regarding controlled substances); contact EOHSS for more information

The use of all expired drugs is prohibited
The use of expired drugs is prohibited for both survival and non-survival procedures (any procedures involving live animals)

Expired non-drug materials -

- Expired non-drug materials can be used only in non-survival studies (1,2)
- All expired materials must be individually labeled as “EXPIRED-ACUTE USE ONLY”
- Expired and non-expired medical materials must be physically separated to avoid confusion

Non-pharmaceutical grade compounds

Consideration of non-pharmaceutical grade compounds should include -

- Lack of acceptable/available veterinary or human pharmaceutical-grade compounds
- Investigation of novel therapeutic drugs
- Specific review and approval by the IACUC
- Grade, purity, sterility, pH, pyrogenicity, osmolality, and stability (1)

Avertin (tribromoethanol) – Use of avertin requires scientific justification. The side effects of avertin include acute inflammatory changes, local irritation, fibrous adhesions in the abdominal cavity, and mortality following one or repeated IP injections. Stock solutions must be kept in dark vials at 4°C and discarded within 2 weeks after preparation (7).

Cost savings alone are not an adequate justification for using non-pharmaceutical-grade compounds in animals
Controlled drugs

Record of use - The use/administration of controlled substances must be recorded every time the drug is administered to an animal. When a controlled drug is incorporated into a drug cocktail (such as ketamine/xylazine, common in rodents), the initial amount of controlled drug used to create the cocktail must be recorded, then an additional log sheet should be created for the cocktail. The cocktail vial should include date of creation, expiration date, concentration of each component, and dosing information. Individual administration of cocktail (volume) should then be recorded. A sample controlled drug log sheet is included in appendix I.

Storage (5) – Controlled substances must be secured within a locked steel cabinet, drawer, or safe that cannot be moved or transported. The storage cabinet must be kept in a room within the laboratory that can be locked as well.

General guidelines -
- Store all drugs in a secure, dedicated location
- Assign responsibilities to one specific individual, with another individual as backup
- Establish an inventory system that minimizes the amount of drug or medical supplies on hand
- Perform regular monthly checks of inventory and discard all expired drugs or medical materials following federal and state regulations; contact EOHSS for drug disposal (6)

References -

1. OLAW: Q &A Expired drugs: [http://grants.nih.gov/grants/OLAW/faqs.htm#useandmgmt_5](http://grants.nih.gov/grants/OLAW/faqs.htm#useandmgmt_5)

(Appendix 1 on proceeding page)
Appendix 1 – Sample controlled substance log

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Lot Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration of Drug</td>
<td>Total quantity supplied</td>
</tr>
<tr>
<td>Expiration Date</td>
<td>Date Issued</td>
</tr>
<tr>
<td>Principal Investigator</td>
<td>Research Staff Signature</td>
</tr>
<tr>
<td>TRC Staff Initials</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Date</th>
<th>Protocol Number</th>
<th>Species/Animal Number</th>
<th>Amount Used</th>
<th>Balance</th>
<th>Initials</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

For disposal only

TRC Signature

Date Returned
Policy 12 - Use of Recording Devices in the Animal Facility

Version 1.0
Approval Date: 6/27/12

Background: The following policy is intended to ensure a minimally disruptive environment for resident animals, to protect the health of research animals, to protect the confidentiality and integrity of research, and to help in the accurate representation of the University’s policies and procedures. Photography or videotaping is only allowed after prior approval by the Vivarium Director or Associate Director. Photography is defined as all still pictures (digital or film) and video recording (digital or analog).

Definitions:

Devices: Cameras (digital or film), video recorders, camera phones, tablet computers, laptops, and similar devices with recording capabilities.

General considerations:

1. The recording devices must be sanitized prior to Vivarium entry according to Vivarium recommendation.
2. The photograph should show appropriate and accurate context (e.g. if an animal is anesthetized or sedated, include the vaporizer or tray holding the bottle of injectable drug).
3. All attempts should be made to have animals in clean surroundings - clean cages or clean pens with clean accessories. Water bottles and feeders should be full if visible in the photo.
4. IACUC approved policies and guidelines must be followed.
5. No references to personal information should be visible in the photograph. Pay attention to background and items such as cage cards.
6. Pictures of personnel require approval of each individual photographed.
7. All persons in the photograph must wear appropriate personal protective equipment.
8. Appropriate handling and restraint methods for the species must be used.
9. Pictures must be downloaded into a secure computer and deleted from the recording devices before leaving the University.
10. Encrypted file transfer must be used for picture dissemination (i.e. Large File Transfer Service (LiFT)).

Research staff:

1. Twenty-four-hour advance notice must be given to the Vivarium Supervisors.
2. Requests may be denied if presented on short notice.
3. All procedures shown must be described in the approved IACUC protocol for that particular animal.
4. No animals that are ill, have visible lesions, or visible research alterations (implants, tumors, etc.) are to be photographed unless approved by the Vivarium Director or Associate Director and the photography is required for scientific publication and/or data analysis by the PI.
**Vivarium veterinary staff:**
1. Photography is for educational seminars, clinical diagnoses or post-approval monitoring (PAM) only.
2. Only dedicated Vivarium cameras are to be used.
3. The camera must be kept in a secure, locked area in the Vivarium.
4. Pictures must be downloaded onto a secure computer and distributed only by encrypted email (i.e., LiFT).
5. Pictures used for training purposes must not have any reference to the PIs or the facility.

**Visitors:**
1. Visitors are not permitted to take still or video recordings in the animal facility except: (1) is a government inspector and photodocumentation is necessary for official documentation and (2) visitor is serving as a photography vendor for the faculty—all such vendor photodocumentation is subject to the policies listed above and must be approved by the Vivarium Director or Associate Director. Only IACUC-approved procedures can be photographed.
2. The faculty member should advise visitors concerning the prohibition of photography at the time of entrance into an animal facility and in conjunction with any request for a visit.

**Note:**
The Vivarium Director and or Associate Director reserve the right to review any and all pictures and video recordings, tape recordings, or camera (film or digital) images before release, and may require that these images/recordings be destroyed.
Policy 13 - Q Fever and Zoonotic Disease Prevention (Sheep)

Version 1.0
Approval Date: 6/27/12

Background: Q fever is a zoonotic disease caused by the rickettsial organism *Coxiella burnetii*. Cattle, sheep and goats are the most common reservoirs of *C. burnetii* and large numbers of organisms (up to 10^9 organisms per gram of tissue) may be present in placenta, birth tissues and amniotic fluids of infected animals. Human infection usually occurs through inhalation of contaminated dusts and aerosols generated by infected animals, their waste products, placental tissues and fluids, and contaminated straw or bedding. Only 10 inhaled bacteria may be sufficient to cause infection in a susceptible host. Most patients will recover to good health within several weeks without any treatment. Persons at risk (i.e. those with valvular heart disease, persons who are immunosuppressed, pregnant women) should be advised of the risk of serious illness that may result from Q fever.

Sheep requirements:
1. **General health:** Animals are healthy as determined by veterinary staff upon arrival. Sheep are purchased from a flock with no history of Q-fever or Caseous Lymphadenitis (CL). Sick animals are not permitted to enter the animal facility.
2. **Pregnancy:** Only male or non-pregnant females are used. Non-pregnancy is confirmed using serological testing, at least 4 weeks after being isolated from intact males.
3. **Vaccination:** Sheep are vaccinated against: *Clostridium perfringes* types C & D, tetanus toxoid, *Pasteurella haemolytica*, *Pasteurella multiciada*, ovine ecthyma (lesions are healed before delivery), and rabies.
4. **Testing and pathogen exclusion:** Brucellosis and Q fever test results must be negative. Two negative Q fever tests at least 3 weeks apart are required. The second Q fever test must be performed no later than one week before sheep delivery.
5. **Other treatments:** Sheep are dewormed, shorn, receive foot trimming and zinc sulfate treatments.

Operational Practices:
1. **Training:** All personnel involved in sheep studies must attend the sheep zoonotic prevention seminar.
2. **Entry:** The sheep holding area is restricted to personnel who are involved in sheep studies (investigators and animal care staff).
3. **PPE:**
   1) Gloves
   2) Disposable or on site-laundered jumpsuits, coveralls, or scrubs
   3) Knee high boots (when cleaning pens)
   4) **Full face shield or goggles and surgical mask** are worn when cleaning the pens and during cage wash or certain veterinary procedures (i.e., lancing an abscess).
   5) Respiratory protection (N95) or PAPR are required during some veterinary procedures (i.e., lancing an abscess, trimming of foot rot) or for handling sheep with bloody vaginal discharge. Personnel must be enrolled in the Respiratory Protection Program in order to wear a respirator.
4. **Recommended disinfectants:**
   1) Household bleach (diluted to 10%)
   2) Hydrogen peroxide (5%)
   3) Lysol
Policy 14 – Identification of Rodents
Version 1.0
Approval date: 11/14/12

Purpose – The purpose of this policy is to ensure proper identification of each individual and group of rodents in a cage.

Background - Proper identification of research animals is an essential component of a research and is designed and mandated by the Guide. It allows an easy method for tracking animals throughout a research project and assists animal care staff in providing care for animals. The Guide states: “identification cards should include the source of the animal, the strain or stock, names and contact information for the responsible investigator(s), pertinent dates (e.g., arrival date, birth date, etc.), and protocol number when applicable. Genotype information, when applicable, should also be included, and consistent, unambiguous abbreviations should be used when the full genotype nomenclature is too lengthy”.

Policy - Cage cards are used for every rodent cage; additional forms of identification are added to individually identify mice within a cage.

Cage card information:

Based on the Guide’s mandate, UMDNJ requires the following information on every cage card: principle investigator’s name, approved protocol number, name of contact person (can be PI), and contact’s phone number (email address is not acceptable as sole contact information). The PI listed on the cage card MUST be the PI associated with the protocol number. Date of birth must also be included on every cage card for studies using geriatric animals (rodents older than 18 months).

UMDNJ recommends (but does not require) additional information be included on cage cards: source of animals, stock or strain, sex, genotype information (if applicable), and pertinent dates (birth date for non-geriatric animals, arrival date, etc.). UMDNJ recognizes that this information is not always available for every animal.

Individual animal identification:

Temporary Markings: Temporary markings are used short-term for individual animals. Use an indelible marker of varying colors to write numbers, bars, or other distinguishable markings on the tail or the ears. If temporary marking are used for duration exceeding 3-4 days, repeat markings every 3-4 days.

Tattooing: Use an electric tattoo machine to write numbers on the tail using only sterile and sharp tattoo needles; tattooing is easier to perform under general anesthesia. If not using general anesthesia, apply a local anesthetic on the tail before tattooing (EMLA cream or a local anesthetic spray).
**Micro-tattooing:** Use a micro-tattooer or animal lancet to inject tattoo ink in the toe pads and/or the ears. Whenever possible, use a simple identification code to minimize the number of toes tattooed.

**Microchip Transponders:** Microchip transponders are implanted subcutaneously between the scapulae for permanent identification of individual animals. Briefly anesthetize animal; pluck or shave the skin and disinfect with surgical scrub (betadine or chlorhexadine solution). Apply digital pressure with a sterile gauze pad if bleeding is noted after implantation. If necessary, a drop of surgical glue is applied to the needle entry site.

**Ear Tags:** Mice are ear tagged at weaning age or older using tags no more than 5mm in length. Ear tags are rinsed in 70% alcohol before use to help prevent ear infection. Tags are positioned at the lateral base of the ear, approximately 3mm from the edge of the ear pinna. Ear tags are not placed too close to the edge of the pinna or too close to the cartilage at the base of the ear pinna.

Proper location of ear tags:

Monitor the tag implantation site 2-3 times a week for signs of local infection. Contact veterinary staff if any complications occur.

**Ear Punch:** Ear punches are sterilized before use; sanitize the ear punch between each cage of animals with 70% ethanol. The punch is placed approximately 3mm from the edge of the ear pinna:

**Toe clipping:** The *Guide* states: “toe-clipping should be used only when no other individual identification method is feasible. It may be the preferred method for neonatal mice up to 7 days of age as it appears to have few adverse effects on behavior and well-being at this age.” Refer to IACUC policy #9 for further information.

**ANY EXCEPTIONS TO THIS POLICY MUST BE APPROVED BY THE IACUC**
References -


Policy 15 – Mouse Total Body Irradiation
Version 1.0
Approval Date: 11/14/12

Purpose - This policy describes mice exposed to total body irradiation (TBI) emanating from a Cesium 137 source (gamma radiation).

Background - Ionizing radiation causes breaks in the DNA helix, primarily affecting mitotically active cells such as those of the hematopoietic and gastrointestinal tracts. The degree of cellular damage depends on the dose of radiation, age, and strain of the mice. In general, C57Bl/6 mice are more radio-resistant than BALB/c mice. B6 mice can typically tolerate radiation doses of 1000 to 1100cGy, however, the LD50 of BALB/c mice is about 880cGy (Duran-Struuck 2009). Ionizing radiation experiments are most commonly used in the fields of immunology and cancer biology.

Definitions –

- **Gamma irradiation** is one type of ionizing irradiation. Sources are typically Cesium 137, Cobalt 60, or high-energy X-rays; this policy refers to Cesium 137 irradiators.

- **Gray** (Gy) is the SI unit of absorbed radiation.

- **Rad** is a largely obsolete unit of absorbed radiation; 100 Rads = 1 Gray.

- **Fractionation of dose**: The total irradiation dose can be split into two or more equal parts separated by a time interval (usually 2-12hrs) in order to minimize morbidity and mortality.

Policy –

Requirements for animals on study exposed to irradiation -

- Irradiation must be scientifically justified in the IACUC protocol. Animals exposed to radiation must be monitored and findings documented daily on the post-procedural cards for the first 14 days. However, after protocols have been established and PI has experience with the particular strain of mice and dose, three times/week monitoring (with documentation) is acceptable. If animals experience morbidity or mortality daily checks are required.

- The planned dose of irradiation (dose range) and the frequency of the radiation must be specified in the IACUC application.

- Fractionated doses should be considered, if appropriate, to reduce morbidity and mortality.

- Unless literature references are available, a pilot study to determine the best dose is recommended if the PI is starting a new study or using a new strain of mice.

- IACUC policies 3 and 5 for humane endpoints must be followed. However, body weight loss up to 25% is acceptable during the first 2 weeks post-irradiation.

- The irradiation procedure is considered category “E” (USDA classification).
Effects of total body irradiation (TBI):

Irradiated animals experience 5 to 10 days of post-irradiation related sickness. Irradiated mice generally recover within 2-3 weeks (Duran-Struuck 2009).

**Appearance:** Mice may appear lethargic with a rough coat and assume a hunched posture due to radiation-induced tissue damage and inflammatory responses.

**Dehydration:** Early after irradiation mice can become dehydrated due to decreased water consumption and diarrhea that often develops from radiation-induced damage to the intestinal epithelium.

**Body weight loss:** Body weight loss up to 25% due to inappetance and diarrhea peaks at about 7 days post-irradiation. Depending on the dose and whether immune reconstitution had been provided, recovery will usually occur in 2 to 3 weeks. Mice may never regain their original, pre-irradiation body weight.

**Anemia:** Animals may appear pale, especially around the nose and paws.

**Intestinal bleeding:** Dark stool (melena) or blood stained perennial area may be present.

**Infection:** Severe bacteremia/septicemia may occur as a result of translocation of bacteria from the GI tract into the blood stream (Duran-Struuck 2008).

**Graft Versus Host Disease (GVHD):** Successful survival of a bone marrow graft requires suppression of the host’s immune system. If the irradiation dose is too low, Graft Versus Host Disease (GVHD) will ensue. As in humans, older mice are more prone to develop GVHD.

**Graying of hair coat:** Black mice, such as C57BL, will frequently turn gray after irradiation.

**Development of secondary neoplasia:** The development of neoplasia after irradiation has been reported in humans and many large animal species. This may occur in mice on long-term studies as well.

**Incisor damage:** One non-neoplastic illness reported in mice is incisor damage and subsequent difficulty in eating. Giving softened food during the recovery phase is required.

Care of irradiated mice:

**PIs (or designated research staff) are responsible for care of irradiated animals.** Vivarium staff will provide special care in an emergency and in such a case the PI will be charged accordingly. For the first 14 days animals must be checked daily or 3X/week, with their condition and care documented on the rodent post-procedure monitoring card (Blue cards). Care, especially during the first week, must ensure the animals are as comfortable as possible. This includes keeping them clean, hydrated, and having ready access to moistened food and Napa Nectar, if indicated. BCS or body weight
measurement should be performed and recorded in the Blue cards until mice return to normal condition, usually within 2-3 weeks.

**Use of antibiotics in the drinking water** - Administration of antibiotics in the drinking water may minimize bacterial contamination within the water source and potentially decrease the burden of gastrointestinal bacteria. Bacterial translocation from the intestinal tract after irradiation is a common source of systemic infection. PI is responsible for placing rodents on antibiotic water a few days before the scheduled irradiation in order for the animals to acclimate to the taste. Rodents are kept on antibiotic water for at least 14 days and up to 28 days post-irradiation.

**Making drinking water readily available** - Irradiated mice will suffer from radiation sickness and will not feel well for the first 7-14 days. It is important to provide easy access to water.

**Napa Nectar** must be provided on the bottom of the cage during the first 14 days if morbidity or mortality is observed. Napa Nectar is available in the animal room free of charge. Placing a new Napa Nectar in the cage daily can be done by research staff or by Vivarium staff for a fee. Napa Nectar becomes contaminated with fecal material quickly and must be replaced daily.

**Provision of softened food** - Giving softened food during the recovery phase is required. Powdered chow is available in the rodent housing rooms and should be mixed with water and served in a small Petri dish on the cage floor. Pellets moistened with water dry up easily and are not recommended. Placing a new moistened food dish in the cage must be done by the PI (or research staff) daily or can be done by Vivarium staff for a fee.

**Housing** - It is important to realize that even after bone marrow transplantation, lethally irradiated mice are severely immunosuppressed for the first two weeks and providing a completely sterile environment (cage, food, and water) is recommended (Fox 2007, Duran-Struuck 2009) and is required if post-irradiation complications occur.

**Recommended antibiotic drugs and preparation (for water bottles):**

<table>
<thead>
<tr>
<th>drug</th>
<th>stock conc</th>
<th>dose</th>
<th>recipe</th>
<th>frequency</th>
<th>duration</th>
<th>final conc in bottle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>50mg/ml</td>
<td>134mg/kg/day</td>
<td>2.6ml stock + 250ml H₂O</td>
<td>Change bottle every 3 days</td>
<td>14-28 days</td>
<td>0.52mg/ml</td>
</tr>
<tr>
<td>Baytril</td>
<td>22.7mg/ml</td>
<td>40mg/kg/day</td>
<td>1.7ml stock + 250ml H₂O</td>
<td>Change bottle every 3 days</td>
<td>14-28 days</td>
<td>0.15mg/ml</td>
</tr>
<tr>
<td>Sulfamethoxazole-Trimethoprim (SMX-TMP)</td>
<td>40/8 mg/ml</td>
<td>220/42 mg/kg/day</td>
<td>5.2ml stock + 250ml H₂O</td>
<td>Change bottle every 3 days</td>
<td>14-28 days</td>
<td>0.82 / 0.16 mg/ml</td>
</tr>
</tbody>
</table>
References –

1. Boston University policy for Irradiation of Rodents:  
   http://www.bu.edu/orccommittees/iacuc/policies-and-guidelines/irradiation-of-rodents


4. RWJMS IACUC Policies 3 and 5
Purpose – “The primary aim of environmental enrichment is to enhance animal well-being by providing animals with sensory and motor stimulation, through structures and resources that facilitate the expression of species typical behaviors and promote psychological well-being through physical exercise, manipulative activities, and cognitive challenges according to species-specific characteristics.” (the Guide)

Social needs – “An appropriate housing space or enclosure should also account for the animals’ social needs. Social animals should be housed in stable pairs or groups of compatible individuals, unless they must be housed alone for experimental reasons or because of social incompatibility.” (the Guide)

Species-specific enrichments:
- Rats: social housing, wooden chew sticks, sheltering products, edible rewards
- Mice: social housing, nesting materials, sheltering products, edible rewards
- Swine: social housing, manipulable toys, edible rewards, scratching boards
- Sheep: social housing, edible rewards
- Rabbits: social housing, manipulable toys, edible rewards
- Hamsters: wooden chew sticks, sheltering products, edible rewards
- Frogs: sheltering products
- Zebrafish: social housing, edible rewards, sheltering products

Policy: Social animals should be group housed. At least one species-specific enrichment object should be placed inside the animals’ primary environment. Any exceptions must be submitted to the IACUC for approval prior to implementation.

Exemption examples:
1. Interference with experimental results (e.g., enrichment in neurological studies)
2. Animal territorial fighting
3. Paralyzed animals
4. Study requirement for individual food uptake or urine/feces collection
5. Post-op recovery

ANY EXCEPTIONS TO THIS POLICY MUST BE PREAPPROVED BY THE IACUC

References:
   http://gr8tt.com/flipbooks/uniflip_ER_0711%20Folder/uniflip.swf
Policy 17- Personal Protective Equipment (PPE) Requirement for In Vivo Studies

Version 1.0
Approval date: 12/12/12

Purpose: “The use of good personal hygiene will often reduce the possibility of occupational injury and cross contamination.” (the Guide)

Abbreviations: Personnel Protective Equipment (PPE), Child Health Institute (CHI), New Research Tower Building (aka RT-2, SPH, or NRB), Research Tower Barrier (RTA), Cancer Institute of New Jersey (CINJ), Research Tower Building (aka RT-1 or RTB), Medical Education Building (MEB).

Policy:

PPE requirement:

<table>
<thead>
<tr>
<th>Facility</th>
<th>Use of biosafety cabinet (class II)</th>
<th>Required PPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHI,SPH,CINJ, RTA</td>
<td>required</td>
<td>gloves, shoe covers, dedicated lab coat</td>
</tr>
<tr>
<td>CHI suite D</td>
<td>required</td>
<td>gloves, shoe covers, dedicated lab coat, surgical mask, bouffant bonnets</td>
</tr>
<tr>
<td>MEB, RT-1</td>
<td>recommended</td>
<td>gloves, lab coats</td>
</tr>
</tbody>
</table>

Necropsy: Facility-specific PPE must be used. If the procedure is performed outside of the biosafety cabinet, use of a fit-tested respirator is recommended.

Handling dirty cages: Facility-specific PPE must be used. The microisolator top should be placed on the cage(s) before transferring outside of the biosafety hood; use of a fit tested respirator is recommended.

Use of biohazard and chemical hazards: Animals that are treated with chemical or biochemical hazards must be treated according to the EOHSS recommendations.

ANY EXCEPTIONS TO THIS POLICY MUST BE APPROVED BY THE IACUC

References:

Policy 18 – Monoclonal Antibody Production and Ascites

Version 1.0
Approval Date: 12/12/12

Purpose – The purpose of this policy is to provide direction and reference material regarding the production of monoclonal antibodies (MAb) in rodents. This policy does not apply to MAb production in other species such as the rabbit.

Background - The production of MAb in mice involves immunizing the animal, selecting antibody producing cells (B cells), fusing the B cells with myeloma cells, creating an ascites-producing hybridoma, and finally injecting hybridoma cells into primed mice. The NIH concurs with the findings and recommendations in the 1999 Report of the National Research Council Monoclonal Antibody Production\(^1\) which indicates that during the accumulation of ascites fluid there is likely to be pain and distress, particularly when some cell lines that are tissue-invasive are used, and in situations of significant ascites development. The report concludes that there is, and will continue to be, scientific necessity for this method. However, as tissue-culture systems are further developed, tissue-culture methods for the production of monoclonal antibodies should be adopted as the routine method, unless there is a clear reason why they cannot be used. Accordingly, IACUCs are expected to critically evaluate proposed uses of the mouse ascites method by investigators. Prior to approval of such protocols, IACUCs must determine that (i) the proposed use is scientifically justified, (ii) methods that avoid or minimize discomfort, distress, and pain (including \textit{in vitro} methods) have been considered, and (iii) the latter have been found unsuitable \(^2\).

Policy –

\textit{In vitro} methods: \textit{In vitro} methods must be considered first. Refer to the Cornell University website for a list of commercial sources for \textit{in vitro} production of monoclonal antibodies \(^4\).

1. \textit{In vivo} antibody production\(^1\): Refer to IACUC Fluid Policy #19 for acceptable fluid volumes and needle sizes for injections.

The use of \textit{in vivo} MAb production requires scientific justification, examples of such include:
1.1. Some hybridoma cell lines do not adapt well to \textit{in vitro} conditions.
1.2. Monoclonal antibodies from mouse ascites fluid might be essential for experiments in which MAb are used in mice.
1.3. Rat hybridoma cell lines do not generate ascites efficiently in rats, usually adapt poorly to \textit{in vitro} conditions, but usually generate ascites in immuno-compromised mice.
1.4. Downstream purification can lead to protein denaturation and decreased antibody activity.
1.5. Serum-free or low-serum conditions cannot provide sufficient amounts of MAb for some purposes, such as the evaluation of new vaccines against infectious organisms.

63
1.6. Culture methods sometimes yield populations of IgG MAb that are glycosylated at positions different from those harvested from mouse ascites fluid, thereby influencing antigen-binding capacity and important biologic functions.

1.7. When hybridoma cells producing MAb are contaminated with infectious agents (such as yeast or fungi), the cells often must be passed through mice.

2. **Immunization procedure:** Less toxic, alternative adjuvants to Complete Freund’s Adjuvant (CFA) should be used; the use of CFA requires scientific justification. Refer to IACUC policy #20 regarding proper use of CFA in rodents. CFA/antigen mixtures should be limited to primary immunization and Incomplete Freund’s Adjuvant (IFA) should be used in subsequent booster inoculations. Refer to IACUC Fluid Policy #19 for proper needle size and injection volumes.

3. **Priming agents:** Priming agents to promote ascites are generally administered IP prior to inoculation of hybridoma cells. Priming of the peritoneal cavity is often accomplished through an IP injection of pristine; ≤0.20 ml should be delivered.

4. **Induction of hybridoma cells:** Hybridomas should be tested for the presence of adventitious viral and mycoplasma agents prior to inoculation into mice in order to prevent potential transmission of murine infectious agents into animal facility experimental colonies. Refer to IACUC policy #6 for further information.

5. **Ascites:** The cranial displacement of the diaphragm due to ascites is associated with dyspnea, orthopnea, or tachypnea. It is therefore reasonable to assume that mice with large accumulations of ascites fluid experience discomfort and distress\(^1\). There is a limit of 3 abdominal taps per animal (two taps in live animals and a final tap after euthanasia); an 18-22 gauge needle should be used. General anesthesia is recommended during tapping. 1-2ml of warm (~37°C) saline should be administered subcutaneously to help prevent shock post tap. Body weight of mice should not exceed 20% of the normal weight of age- and sex-matched animals of the same strain from the onset of ascites.

6. **Clinical Signs:** Animals must be observed for signs of distress and pain. Clinical signs include: rapid or labored breathing, pallor, hunched posture, inactivity, dehydration, inappetance, low body condition score (BCS), rough hair coat, ambulation difficulty, constipation, or diarrhea. Animals that show signs of excessive distress or appear debilitated after any of the taps should be given fluids or euthanized. The veterinary staff should be contacted for immediate evaluation.

7. **Frequency of observations:** Animals must be evaluated every other day during the first post-inoculation week. However, once ascites fluid accumulation and peritoneal cavity distention is noted, daily observation (including weekends and holidays) of animals is required.
8. **Humane endpoints:** Animals must be euthanized when the following symptoms are observed: prolonged [inappetance, inactivity, diarrhea/constipation, hunched posture, rough coat], hypothermia, tachypnea, labored breathing, pallor, inability to remain upright, or any other clinical signs indicated in IACUC Policies #1,3, 5, or veterinary staff recommendation.

9. **Summary of Ascites production:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid volume, site of injections, needle sizes</td>
<td>IACUC Fluid Policy #19 (recommendations)</td>
</tr>
<tr>
<td>Testing cell lines for murine viruses</td>
<td>IACUC Policy #6 (required)</td>
</tr>
<tr>
<td>Priming</td>
<td>pristane, 0.20 ml IP</td>
</tr>
<tr>
<td>Needle size for tap</td>
<td>18-22 gauge</td>
</tr>
<tr>
<td>Number of taps</td>
<td>Maximum of three (3rd after euthanasia)</td>
</tr>
<tr>
<td>Fluid volume administered</td>
<td>IACUC Fluid Policy 19</td>
</tr>
<tr>
<td>Monitoring after hybridoma inoculation</td>
<td>3 times a week during the first week, then daily</td>
</tr>
<tr>
<td>CFA use</td>
<td>Need scientific justification (only one CFA injection per animal), IACUC Policy 20</td>
</tr>
<tr>
<td>Fluid replacement after ascites harvesting</td>
<td>1-2 ml warm saline SC</td>
</tr>
<tr>
<td>General anesthesia during tap</td>
<td>Recommended to prevent pain and distress</td>
</tr>
<tr>
<td>Humane endpoints</td>
<td>IACUC Policies 1,3, and 5</td>
</tr>
</tbody>
</table>

**References**

Policy 19 – Fluid Administration and Collection in Rodents

Version 1.0
Approval Date: 12/12/12

Purpose – This policy was designed to provide investigators with reference values related to the administration and collection of fluids (including blood) in rodents via the most common experimentally used routes.

Background – The values included in this policy are cited directly from the literature, represent an average of multiple cited sources, or based on personal experience of the Veterinary Staff. This policy contains three sections: the first section provides recommendations regarding dosing of compounds based on common routes of administration. Included are typical injection volumes and maximum allowable volumes for each route. The second section of the policy includes common sites of blood collection, along with expected volumes for each site/method. The final section (section 3) reviews injection of tumors/pellets through a trocar (defined as any needle greater than 16ga).

Definitions:

The following abbreviations will be used in this policy:

- PO = per os (oral, gavage)
- IM = intramuscular
- IN = intranasal
- IV = intravenous
- SC = subcutaneous
- IT = intrathecal (into subarachnoid space of spinal column)
- ID = intradermal
- EP = epidural (outside of meninges)
- IP = intraperitoneal
- ICV = intracerebroventricular (into lateral ventricle of brain)
Section 1: Fluid Administration

Vehicle selection is an important consideration in compound administration. Ideally, the vehicle should be biologically inert and have no toxic effect on the animal. Osmolality, pH and viscosity of the vehicle should be considered when preparing compounds. If possible, compounds should be prepared so that the delivery volume is close to the typical volume (value on left side under body weight on chart). Note that compounds cannot be delivered in a volume greater than the maximum valued listed on the right column under body weight (highlighted in red) without prior IACUC approval.

Mice (values across top are body weights in grams)

<table>
<thead>
<tr>
<th>route</th>
<th>10</th>
<th>15</th>
<th>20</th>
<th>25</th>
<th>30</th>
<th>≥35</th>
<th>needle</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>100</td>
<td>500</td>
<td>150</td>
<td>750</td>
<td>200</td>
<td>1000</td>
<td>250</td>
</tr>
<tr>
<td>IN</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
</tr>
<tr>
<td>SC</td>
<td>70</td>
<td>400</td>
<td>105</td>
<td>600</td>
<td>140</td>
<td>800</td>
<td>175</td>
</tr>
<tr>
<td>ID</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>50</td>
</tr>
<tr>
<td>IP</td>
<td>150</td>
<td>800</td>
<td>225</td>
<td>1200</td>
<td>300</td>
<td>1600</td>
<td>375</td>
</tr>
<tr>
<td>IM</td>
<td>1.5</td>
<td>2</td>
<td>2.25</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>3.75</td>
</tr>
<tr>
<td>IT</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>40</td>
<td>10</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>ICV</td>
<td>5</td>
<td>20</td>
<td>10</td>
<td>40</td>
<td>10</td>
<td>40</td>
<td>10</td>
</tr>
<tr>
<td>IV (bolus)</td>
<td>50</td>
<td>75</td>
<td>100</td>
<td>125</td>
<td>150</td>
<td>175</td>
<td>≥25ga</td>
</tr>
<tr>
<td>IV (slow)</td>
<td>250</td>
<td>375</td>
<td>500</td>
<td>625</td>
<td>750</td>
<td>875</td>
<td>≥25ga</td>
</tr>
</tbody>
</table>

All values listed on chart are in MICRO liters (µL); value on left is typical volume, volume on right (highlight in red) is the maximum volume allowed by that route. * = requires IACUC approval

Rats (values across top are body weights in grams)

<table>
<thead>
<tr>
<th>route</th>
<th>100</th>
<th>150</th>
<th>200</th>
<th>250</th>
<th>300</th>
<th>350</th>
<th>400</th>
<th>450</th>
<th>500</th>
<th>needle</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO</td>
<td>1</td>
<td>4</td>
<td>1.5</td>
<td>6</td>
<td>2</td>
<td>8</td>
<td>2.5</td>
<td>10</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>IN</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
<td>35</td>
<td>50</td>
</tr>
<tr>
<td>SC</td>
<td>0.5</td>
<td>1</td>
<td>0.75</td>
<td>1.5</td>
<td>1</td>
<td>2</td>
<td>1.25</td>
<td>2.5</td>
<td>1.5</td>
<td>3</td>
</tr>
<tr>
<td>ID</td>
<td>0.05</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>0.05</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>IP</td>
<td>1</td>
<td>2</td>
<td>1.5</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>2.5</td>
<td>5</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>IM</td>
<td>0.01</td>
<td>0.02</td>
<td>0.015</td>
<td>0.03</td>
<td>0.02</td>
<td>0.04</td>
<td>0.025</td>
<td>0.05</td>
<td>0.06</td>
<td>0.035</td>
</tr>
<tr>
<td>EP</td>
<td>0.015</td>
<td>0.023</td>
<td>0.003</td>
<td>0.03</td>
<td>0.04</td>
<td>0.038</td>
<td>0.05</td>
<td>0.05</td>
<td>0.06</td>
<td>0.053</td>
</tr>
<tr>
<td>IT</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>1</td>
<td>0.01</td>
<td>1</td>
</tr>
<tr>
<td>ICV</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≤0.02</td>
<td>≥28ga</td>
</tr>
<tr>
<td>IV (bolus)</td>
<td>0.5</td>
<td>0.75</td>
<td>1</td>
<td>1.25</td>
<td>1.5</td>
<td>1.75</td>
<td>2</td>
<td>2.25</td>
<td>2.5</td>
<td>≥23ga</td>
</tr>
<tr>
<td>IV (slow)</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
<td>8</td>
<td>9</td>
<td>10</td>
<td>≥23ga</td>
</tr>
</tbody>
</table>

All values listed on chart are in MILLI liters (mL); value on left is typical volume, volume on right (highlighted in red) is the maximum volume allowed by that route.
Notes on specific routes:

**Oral gavage (PO)** is performed using a feeding needle only (has an atraumatic, blunt ball at the end to prevent damage to the esophagus), appropriate needle length is determined by measuring from the mouth to the last rib; inject slowly. Proficient oral gavage should result in no significant animal losses (>95% survival rate).

**Subcutaneous injections** are limited to a maximum of 3 injections every 24hrs; typically SC injections are delivered on the back/dorsum or between the shoulder blades. SC fluids to account for blood/fluid loss (such as given postoperatively) are typically <0.5ml in the mouse and <3ml in the rat.

**IM injections** are limited to the quadriceps femoris or biceps femoris muscle groups. Due to the small size of mice, IM injections are not permitted in mice without prior IACUC approval. No more than two IM injections are permitted every 24hrs.

**IT, ICV** injections should be given over at least 1-2min per 10μl in mice and no greater than 0.25ml per minute in rats.

A **bolus IV injection** is delivered within 1 minute or less; typical IV injection sites in rodents include the lateral tail veins and the saphenous veins.

A **slow IV injection** is delivered over a 5-10 minute period.

Contact the Veterinary Staff for information regarding other routes of administration.

**Section 2: Blood Collection**

**Circulating blood volume (CBV) in rodents is ~55-70 ml/kg (~5.5-7.0% body weight, mouse average = 7.2% BW, rat average = 6.4% BW).** Investigators can safely remove 1% CBV every 24hrs, or 10% CBV every 2-4wks. No more than 20% CBV can be removed at one time and requires sufficient recover time (see below). Animals MUST have appropriate recovery time after collection, based on the total volume of blood removed.

Factors to consider when choosing the best blood collection method should include:

- type of sample (whole blood, serum, etc.)
- quantity of blood required
- quality of sample (sterility, tissue contamination, etc.)
- frequency of sampling
- health status of the animal(s)
- training/experience of collector
Blood sample volume ranges based on body weight:

<table>
<thead>
<tr>
<th>BW (g)</th>
<th>CBV (ml)</th>
<th>1% (ml)</th>
<th>10% (ml)</th>
<th>20% (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1.10-1.40</td>
<td>0.011-0.014</td>
<td>0.11-0.14</td>
<td>0.22-0.28</td>
</tr>
<tr>
<td>25</td>
<td>1.37-1.75</td>
<td>0.014-0.018</td>
<td>0.14-0.18</td>
<td>0.28-0.36</td>
</tr>
<tr>
<td>30</td>
<td>1.65-2.10</td>
<td>0.017-0.021</td>
<td>0.17-0.21</td>
<td>0.34-0.42</td>
</tr>
<tr>
<td>35</td>
<td>1.93-2.45</td>
<td>0.019-0.025</td>
<td>0.19-0.25</td>
<td>0.38-0.50</td>
</tr>
<tr>
<td>40</td>
<td>2.20-2.80</td>
<td>0.022-0.028</td>
<td>0.22-0.28</td>
<td>0.44-0.56</td>
</tr>
<tr>
<td>Rat</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>125</td>
<td>6.88 - 8.75</td>
<td>0.069-0.088</td>
<td>0.69-0.88</td>
<td>1.38-1.76</td>
</tr>
<tr>
<td>150</td>
<td>8.25-10.50</td>
<td>0.082-0.105</td>
<td>0.82-1.0</td>
<td>1.64-2.0</td>
</tr>
<tr>
<td>200</td>
<td>11.00-14.00</td>
<td>0.11-0.14</td>
<td>1.14-1.4</td>
<td>2.2-2.8</td>
</tr>
<tr>
<td>250</td>
<td>13.75-17.50</td>
<td>0.14-0.18</td>
<td>1.4-1.8</td>
<td>2.8-3.6</td>
</tr>
<tr>
<td>300</td>
<td>16.50-21.00</td>
<td>0.17-0.21</td>
<td>1.7-2.1</td>
<td>3.4-4.2</td>
</tr>
<tr>
<td>350</td>
<td>19.25-24.50</td>
<td>0.19-0.25</td>
<td>1.9-2.5</td>
<td>3.6-5.0</td>
</tr>
</tbody>
</table>

Blood collection recovery times:

<table>
<thead>
<tr>
<th>single sample</th>
<th>multiple samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>% CBV removed</td>
<td>recovery period</td>
</tr>
<tr>
<td>0.75%</td>
<td>24hrs</td>
</tr>
<tr>
<td>7.5%</td>
<td>1 week</td>
</tr>
<tr>
<td>10%</td>
<td>2 weeks</td>
</tr>
<tr>
<td>15-20%</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

Blood collection sites in rodents:

<table>
<thead>
<tr>
<th>mouse (25g)</th>
<th>orbital</th>
<th>yes</th>
<th>limited</th>
<th>5% CBV</th>
<th>mod/high</th>
<th>device/needle</th>
</tr>
</thead>
<tbody>
<tr>
<td>mandibular/cheek (adults only)</td>
<td>no</td>
<td>yes</td>
<td>0.2-0.4ml</td>
<td>mod</td>
<td>lancet, 3-5mm</td>
<td></td>
</tr>
<tr>
<td>saphenous</td>
<td>no</td>
<td>yes</td>
<td>5% CBV</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>lateral tail vein</td>
<td>no</td>
<td>yes</td>
<td>0.1-0.15ml</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>ventral tail artery</td>
<td>no</td>
<td>yes</td>
<td>0.1-0.2ml</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>cardiac</td>
<td>yes/terminal</td>
<td>no</td>
<td>50% CBV</td>
<td>mod</td>
<td>23ga</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>rat (300g)</th>
<th>orbital</th>
<th>yes</th>
<th>limited</th>
<th>5% CBV</th>
<th>mod/high</th>
<th>device/needle</th>
</tr>
</thead>
<tbody>
<tr>
<td>mandibular/cheek</td>
<td>no</td>
<td>yes</td>
<td>0.2-0.5ml</td>
<td>mod</td>
<td>lancet, 5-8mm</td>
<td></td>
</tr>
<tr>
<td>sublingual</td>
<td>yes</td>
<td>yes</td>
<td>0.2-1ml</td>
<td>low</td>
<td>23-25ga</td>
<td></td>
</tr>
<tr>
<td>jugular</td>
<td>no</td>
<td>limited</td>
<td>5% CBV</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>saphenous</td>
<td>no</td>
<td>yes</td>
<td>5% CBV</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>lateral tail vein</td>
<td>no</td>
<td>yes</td>
<td>up to 2ml</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>ventral tail artery</td>
<td>no</td>
<td>yes</td>
<td>0.1-0.2ml</td>
<td>low</td>
<td>25-27ga</td>
<td></td>
</tr>
<tr>
<td>cardiac</td>
<td>yes/terminal</td>
<td>no</td>
<td>50% CBV</td>
<td>mod</td>
<td>23ga</td>
<td></td>
</tr>
</tbody>
</table>
Notes on specific techniques:

**Mandibular** samples will contain a mixture of venous and arterial blood.

Blood from the **saphenous** and **tail veins** can be achieved either by introducing an appropriate needle into the vessel, or by nicking the vessel and collecting blood into a container; note that samples collected by the latter method will not be sterile and could be contaminated with tissue(s).

**Lateral tail vein:** Prewarming the tail under a heat lamp or local warming will cause vasodilation, increasing yield.

**Orbital (retrobulbar)** samples are collected with a heparinized capillary tube from the medial/rostral canthus of the eye only; samples can be taken at a maximum of every 2 weeks from the same site. Personnel must be adequately trained in technique to avoid injury to the animal; technique is less commonly performed in the rat (rat has plexus while mouse has sinus).

**Cardiac blood sampling** is only permitted as a terminal procedure in a deeply anesthetized animal.

**Section 3: Tumor/Pellet Delivery by Trocar**

Pieces of tumor and pellets (often for slow release of drugs or hormones) are usually implanted subcutaneously in rodents through a large-bore needle called a trocar. Because of the large diameter of a trocar (≤16ga), more than momentary pain is associated with their use; therefore, all procedures involving trocars are considered minor survival surgery at by the IACUC. Animals MUST be under general anesthesia or have a local anesthetic agent applied at the trocar site for these procedures. Trocars will cause more damage to the skin compared to smaller gauge hypodermic needles, and skin closure (suture, staples, wound clips, surgical glue) may be needed post injection. Additionally, animals should be provided with analgesia for at least 12hrs postoperatively.

Common sites of tumor/pellet insertion include the lateral flank (just in front of the hip) and the interscapular area of the dorsum (between the shoulder blades). The IACUC recommends injection of tumors at the lateral flank to reduce irritation from the overlying wire insert of the cage (can irritate developing tumors on the dorsum). For recommendations regarding other acceptable areas, please contact the Veterinary Staff.
References -


Purpose – The purpose of this policy is to provide guidance and resources for investigators that require adjuvant use in live animals.

Background – Adjuvants include any compound that enhances the immune response to an antigen. Adjuvants are commonly used for the in vivo production of polyclonal antibodies either to foreign or self antigens. Many adjuvants are commercially available, and selection is based on intended use and desired effect. Examples include vaccine development/use (low immune response), monoclonal/polyclonal antibody production and collection (moderate immune response), and induction of autoimmune disease (intense immune response). No adjuvant is ideal for all situations and all adjuvants produce varying undesirable side effects, including toxicity.

Commonly used adjuvants:

Complete Freund’s Adjuvant (CFA) – Water-in-oil immersion containing heat-killed Mycobacterium tuberculosis and/or mycobacterial cell wall components; CFA induces a very strong inflammatory response at the injection site that can be painful to the animal. Repeated use can produce sterile abscesses, skin ulceration, and skin/tissue sloughing. CFA is typically only given for the initial immunization, followed by boosters of IFA.

Incomplete Freund’s Adjuvant (IFA) – Similar preparation to CFA, except IFA lacks the Mycobacterium tuberculosis component. Because IFA is less inflammatory, it can be used multiple times in the same animal safely.

Other commercially available adjuvants include RIBI®, TiterMax®, Specol®, montamides, SAF, aluminum compounds, MF59, liposomes, and others.

Policy –

All adjuvants/antigens must be prepared using sterile technique. The preferred route of administration for most adjuvants is subcutaneous (SC).

Antigen/adjuvant injection site(s) should be aseptically prepared, including shaving of site followed by disinfection with surgical scrub.

CFA should be the last resort regarding adjuvant choice; its use requires scientific justification along with demonstration of a search for alternative adjuvants (databases such as ALTWEB or ALTBIB) for IACUC approval.

- animal protocols using CFA are automatically classified at as IACUC Category 3 (equivalent of USDA Category E)
- CFA is only allowed to be administered to each animal once (usually initial immunization)
- CFA should be prepared 1:1 (volume) with aqueous antigen
- if possible, prepare concentrations of CFA <0.1mg/ml (may not be possible for auto-immune disease induction)
- inject volume at multiple sites to minimize inflammation and avoid fusion of lesions

Recommended volumes/sites for CFA-antigen emulsion administration (all volumes in microliters, mls) [1]:

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>ID</th>
<th>IP</th>
<th>FP</th>
<th>IM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>&lt;0.1</td>
<td>*</td>
<td>&lt;0.2*</td>
<td>&lt;0.05**</td>
<td>&lt;0.05***</td>
</tr>
<tr>
<td>Rat</td>
<td>&lt;0.1</td>
<td>&lt;0.05**</td>
<td>&lt;0.5**</td>
<td>&lt;0.1**</td>
<td>&lt;0.1***</td>
</tr>
<tr>
<td>Rabbit</td>
<td>&lt;0.25</td>
<td>&lt;0.05**</td>
<td>*</td>
<td>*</td>
<td>&lt;0.25***</td>
</tr>
</tbody>
</table>

SC = subcutaneous, ID = intradermal, IP = intraperitoneal, FP = foot pad, IM = intramuscular
* = not recommended ** = requires justification *** = only one limb, requires justification

Post injection care – Post injection monitoring and care is required for all in vivo adjuvant use. The injection site should be monitored for at least three weeks (3 times per week) or until all lesions have healed. Lesions that ulcerate, necrose, or slough must be treated under the direction of the veterinary staff. Animals that show overt signs of pain (hunched appearance, poor coat, discharge around eyes, etc.) should receive analgesics (check with veterinary staff regarding choice) [3].

References

Policy 21 – Investigator Responsibilities Regarding Animal Care and Use

Version 1.0
Approval Date: 1/16/13

Purpose – The purpose of this policy is to summarize all responsibilities of investigators using animals at the Robert Wood Johnson Medical School (RWJMS).

Background – The most recent edition of the Guide (8th ed.) includes 29 additional “must” statements as compared to the 7th edition (1996). Many of these statements relate directly to institutional animal care and use. In order to ensure animal welfare and prevent confusion, the IACUC has created an outline of responsibilities investigators assume when working with live animals at RWJMS.

Policy –

All investigators are required to:

1. Attend Translational Resource Center (TRC) access training seminar before working with animals.
2. Have approved protocol(s) accurately describing all animal work performed; PI must have approval letter from IACUC before beginning experiments.
3. Not deviate from IACUC approved protocol(s).
4. Read and adhere to all pertinent IACUC policies and guidelines (see appendix 1).
5. Review and sign their IACUC application(s), amendments, and annual reviews.
6. Provide current contact information (email and phone number) for at least one lab member (does not have to be PI); this allows veterinary staff to communicate with lab regarding after hours/weekend emergencies.
7. Regularly communicate with TRC staff regarding animal health and humane endpoints.
8. Follow recommendation of the veterinary staff regarding treatment of sick/moribund animals.
9. Amend all protocol changes to the IACUC; PI must have written approval prior to initiation of changes (appendix 2 contains common examples of changes that require IACUC approval).
10. Assure all lab personnel have adequate skills to perform in vivo procedures.
11. Report all new phenotypes (trait expression) that affect the animal’s health and welfare to the IACUC and veterinary staff.
12. Assure proper record-keeping of animals (such as completing Blue cards) for procedures that produce pain and distress. This includes (but is not limited to) all major (body cavity entered) surgery. Examples are: post-op care, prolonged restraint, animal models involving paresis/paralysis, animals under veterinary treatment (such as ulcers), and animals approaching humane endpoints (tumor size 1cm (mice), BCS=2, inability to eat/drink, etc.).
13. Monitor all animals under protocol(s) at least 3X/week (or more) as described in the IACUC protocol or veterinary recommendations.
14. Report any observed non-compliance (within your laboratory or other laboratories) to IACUC members or the veterinary staff.
15. Provide a copy of approved protocol(s) to any lab member using live animals.
16. Assure all laboratory members follow this policy.
PIs are ultimately responsible for all animals under their protocol(s)

Additionally, University veterinarians have the authority to treat or euthanize any animal in the interest of animal welfare without prior authorization from the PI. Veterinarians will, however, make every attempt to contact the PI (or laboratory designee) before treating/euthanizing any animal(s).

“… the veterinarian must have the authority, delegated by senior administration and the IACUC, to treat the animal, remove it from the experiment, institute appropriate measures to relieve severe pain or distress, or perform euthanasia if necessary.”

-the Guide, p114

“The veterinarian must have authority to use appropriate treatment or control measures, including euthanasia if indicated, following diagnosis of an animal disease or injury. If possible, the veterinarian should discuss the situation with the principal investigator to determine a course of action consistent with experimental goals. However, if the principal investigator is not available, or if agreement cannot be reached, the veterinarian must have authority to act to protect the health and well-being of the institutional animal colony. The veterinarian's authority should be exercised with the concurrence of the IACUC and the Institutional Official.”

-Guidelines for Adequate Veterinary Care (ACLAM 1996)

Appendix 1 – Current policies at RWJMS

<table>
<thead>
<tr>
<th>#1</th>
<th>Tumors</th>
<th>#12</th>
<th>Use of Recording Devices in Animal Facilities</th>
</tr>
</thead>
<tbody>
<tr>
<td>#2</td>
<td>Overcrowding</td>
<td>#13</td>
<td>Q Fever and Zoonotic Disease Prevention in Sheep</td>
</tr>
<tr>
<td>#3</td>
<td>Experimental Endpoints</td>
<td>#14</td>
<td>Identification of Rodents</td>
</tr>
<tr>
<td>#4</td>
<td>Euthanasia</td>
<td>#15</td>
<td>Mouse Total Body Irradiation</td>
</tr>
<tr>
<td>#5</td>
<td>Weight Loss</td>
<td>#16</td>
<td>Social Housing and Environmental Enrichment</td>
</tr>
<tr>
<td>#6</td>
<td>Use of Cell Lines</td>
<td>#17</td>
<td>Personal Protective Equipment</td>
</tr>
<tr>
<td>#7</td>
<td>Care of EAE Mice</td>
<td>#18</td>
<td>Monoclonal Antibody Production and Ascites</td>
</tr>
<tr>
<td>#8</td>
<td>Genotyping of Rodents</td>
<td>#19</td>
<td>Fluid Administration and Collection (Rodents)</td>
</tr>
<tr>
<td>#9</td>
<td>Toe Clipping</td>
<td>#20</td>
<td>Use of Adjuvants</td>
</tr>
<tr>
<td>#10</td>
<td>Survival Surgery</td>
<td>#21</td>
<td>Investigator Responsibilities</td>
</tr>
<tr>
<td>#11</td>
<td>Expired / Non-Commercial Drugs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Appendix 2 – Common changes to a protocol that requires an amendment [2]:

- Change(s) in objective(s) of study
- Switch from non-survival to survival surgery
• Change(s) in degree of invasiveness of a procedure or discomfort to an animal
• Change in species or number of animals used
• Change in personnel involved in animal procedures
• Change in anesthetic agent(s) or in the use/withholding of analgesics
• Change in method(s) of euthanasia
• Change in duration, frequency, or number of procedures performed on an animal
• Incorporation/change of use of hazardous substances (chemical, biological, radioactive, carcinogens)
• Any exception(s) to federal regulations (the Guide or Animal Welfare Act) and/or IACUC policies

References -


Policy 22 – Animal Welfare Concern Reporting (Whistle Blower)

Version 1.0
Approval Date: 3/20/13

Purpose - Robert Wood Johnson Medical School (RWJMS) is committed to the humane treatment of all animals used in research, testing, teaching and production. The privilege to use live animals for the advancement of science and medicine carries with it the responsibility to follow all applicable laws, policies, and procedures concerning animal welfare, as developed by the government and RWJMS.

Background –

“The institution must develop methods for reporting and investigating animal welfare concerns, and employees should be aware of the importance of and mechanisms for reporting animal welfare concerns.”

- the Guide

“No facility employee, committee member, or laboratory personnel shall be discriminated against or subject to any reprisal for reporting violations.”

- AWA (9 CFR Ch.1), Part 2 - Subpart C, 2.32.2

Policy –

To file a complaint:
• Submit a written report of the alleged violation to the Chairperson of the IACUC (Dr. David Crockett)
• The report should include a factual description of date, time, location, animal species, numbers, identification of animals, personnel involved, and any other relevant details

Anonymous reports are also accepted if sufficient detail is provided to allow adequate investigation of the allegations.

The complainant’s identity will be kept confidential according to the federal Whistleblower Law 3.

Contact Information:

Institutional Official
Dr. Celine Gelinas
gelinace@umdnj.edu
732-235-5035

IACUC Chair
Dr. David Crockett
crockett@umdnj.edu
732-235-5153
Attending Veterinarian
Dr. Bruce Scharf
scharfba@umdnj.edu
732-235-4570

References -


2. Animal Welfare Act