Use of Complete Freund’s Adjuvant

**Policy:** Alternatives to Complete Freund’s Adjuvant (CFA) must be considered and utilized if and when possible. If adequate alternatives to CFA are not feasible, undesirable side effects should be reduced or eliminated through the use of appropriate routes of administration, adequate separation of injection sites, and the use of a small amount of inoculum per site.

**Rationale:** The use of CFA may be associated with the potential for pain or distress. Several alternative adjuvants are available, some of which may be associated with fewer side effects (see OACUC 2013 [reference]). The American Veterinary Medical Association views pain induced by inflammatory agents as “an unnecessary and unwanted side effect of the use of inflammatory agents, or as a result of poor technique,” and counsels that the side effects “should not be accepted as a normal event in an immunization procedure.” Accidental inoculation of personnel with CFA can result in sensitization to tuberculin as well as chronic, local inflammation which is poorly responsive to antibiotic therapy. Because of the potential for complications from certain routes of administration of immunizations, the following guidelines have been established.

**Procedures, Guidelines, and Exceptions:**

1. CFA may be used only for the first (priming) dose. Subsequent immunizations should be performed using incomplete Freund's or another adjuvant unless explicitly justified. Re-immunization with CFA is rarely warranted. If approved by the IACUC, an interval of at least three weeks should be given between doses.

2. The inoculum should be free of extraneous microbial contamination. Millipore filtration of the antigen before mixing with the adjuvant is recommended when possible.

3. Injection sites must be cleaned to remove debris that may result in contamination and infection. This is probably the major cause of abscess formation in animals.

4. Separation between inoculation sites adequate to avoid coalescence should be encouraged. The volume injected at each site, locations of sites injected, and number of sites/number of injections should be described in the animal use Proposal.

5. Injections containing CFA should be given subcutaneous (SC), rather than intradermal (ID), intramuscular (IM), intravenous (IV) or intraperitoneal (IP). ID injections frequently result in skin necrosis and sloughing; injected intraperitoneally, it can cause peritonitis. IM injections can result in temporary or permanent lameness. IV injections can cause pulmonary lipid embolism. CFA is associated with adjuvant arthritis. If routes other than SC must be used, the Proposal must contain a strong justification; the recommended volumes of CFA-Antigen Emulsion are summarized in the table below (adapted from OACU 2013):

<table>
<thead>
<tr>
<th>Species</th>
<th>SC (ml)</th>
<th>PREFERRED ROUTE</th>
<th>ID (ml)</th>
<th>IP (ml)</th>
<th>Footpad (ml)</th>
<th>IM (ml)</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>
<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>
<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>*</td>
<td>&lt;0.25</td>
</tr>
</tbody>
</table>

*Not recommended
6. Footpad injection of CFA in rodents is discouraged because animals may develop arthritis, chronic pain and lameness, and secondary infections in the inflamed areas. If this procedure is to be used, it must be described and its use scientifically justified, including documentation that injections in other sites do not produce adequate antibody titers for the specific antigen being used. If used, only one hind foot may be injected and injections must be spaced at 2 week intervals. Animals that have received foot pad injections must be housed on contact bedding rather than wire-bottomed cages.

7. Animals must be observed daily for adverse reactions for at least four (4) weeks or until any associated lesions have resolved. The Proposal must specifically detail clinical signs or behaviors indicating a need for intervention, such as the use of analgesics. Examples may include significant erythema, reluctance to bear weight, inappetence, and body weight loss.

**Reference**