

**PORTLAND VETERANS AFFAIRS MEDICAL CENTER  
SUBCOMMITTEE ON ANIMAL STUDIES (PVAMC SAS)  
ANIMAL CARE AND USE GUIDELINES**

**CHECK ONE:**

- I agree to comply with the following guidelines.
- I have attached written justification for deviation from these guidelines.

\_\_\_\_\_  
Principal Investigator

\_\_\_\_\_  
Date

**GUIDELINES FOR THE USE OF ADJUVANTS IN LABORATORY ANIMALS**

**Background.** Improper or unnecessary use of Freund's complete adjuvant may cause inflammation, induration, or necrosis in laboratory animals. Disseminated granulomas have been reported in lungs, liver, kidney, heart, lymph nodes and skeletal muscle after subcutaneous or intravenous injection in rabbits and rats, with similar results in hamsters, mice and guinea pigs (1). Humans accidentally injected have suffered long-term, painful abscesses (2). The SAS has developed the following guidelines to eliminate or reduce to a minimum animal discomfort associated with the use of this agent.

**Guidelines.**

1. Before using Freund's complete adjuvant, consider the use of the incomplete or another adjuvant (3,4). Use of the RIBI Adjuvant System, the TiterMax from CytRx or other commercially available immunoadjuvants are acceptable alternatives.
2. If complete Freund's must be used:
  - a. It should be used only for the first (priming) antigenic dose. Use of two or more doses of the complete adjuvant is rarely warranted and must be justified in writing in the animal protocol approved for the study. If more than one dose must be used, an interval of at least three weeks should be allowed between doses.
  - b. The inoculum containing the adjuvant should be divided into fractions so that no more than 0.3ml is injected per site for rabbits, 0.1ml for rats, and 0.075ml for mice, or 0.05ml per footpad.
  - c. Injections should be subcutaneous or intradermal. Intradermal injections may result in skin necrosis and sloughing. Use of footpad injections is not recommended and will not be allowed unless justification is provided in writing in the animal protocol approved for the study, indicating that this route is specifically required. Injection of a rear footpad in mice may be permitted if other sites do not produce significant responses. For studies tracing the fate of injected antigens, injections should be made intramuscularly in areas drained by particular lymph nodes such as the axillary or inguinal.

- d. The injection site must be observed by the Principal Investigator or his/her designee a minimum of three times per week for four weeks after each injection. Written documentation of these observations should be maintained. Any abnormalities noted during observations must be reported to the Veterinary Care Staff.
3. The inoculum should be free of extraneous microbial contamination. Millipore filtration of the antigen before mixing with adjuvant is recommended when possible.
4. Injection sites should be clean but need not be aseptically prepared.

#### REFERENCES

1. Schiefer, B. and Stunzi, H.: Pulmonary Lesions in Guinea Pigs and Rats After Subcutaneous Injection of Complete Freund's Adjuvant or Homologous Pulmonary Tissue. *Zbl. Vet. Med. A.* 26: 1-10, 1979.
2. Chapel, H.M. and August, P.J.: Report of Nine Cases of Accidental Injury to Man with Freund's Complete Adjuvant. *Clin. Exp. Immunol.* 34: 358-541, 1976
3. Osebold, J.W.: Mechanisms of Action by Immunologic Adjuvants. *JAVMA* 181:983-987, 1982.
4. Niemi, S. M., Fox, J.G. , Brown, L.R., and Langer, R.: Evaluation of Ethylene-Vinyl Acetate Copolymer As a Non-Inflammatory Alternative to Freund's Complete Adjuvant in Rabbits. *Laboratory Animal Science*, 35:609-610, 1985.