

**COMPLETE FREUNDS ADJUVANT:
SAFE WORK PRACTICES INFORMATION PAGE**

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BACKGROUND

For several decades Freund's adjuvants have been considered the most effective adjuvants available for raising antibodies in test animals. "Complete Freund's Adjuvant" (CFA) contains heat-killed mycobacteria, which is a primary agent responsible for stimulating antibody production, but has also been attributed to a number of undesirable side effects (Jackson and Fox 1995). The undesirable side effects attributed to CFA use include increased pain and suffering and morbidity in inoculated test animals and potentially serious health and safety threats to laboratory staff, animal care workers, and other personnel who may be accidentally exposed to CFA (Scott 2001). Due to the potential health and safety risk to university staff, the [Institutional Biosafety Committee](#) (IBC) has classified CFA as a reportable hazardous chemical that must be reported on all Institutional Animal Care and Use Committee (IACUC) protocols.

PURPOSE

The purpose of this page is to provide the research community sufficient information regarding specific health threats/exposure routes, the implementation of proper work methods, and provision of suitable personal protective equipment for development of research protocols that include measures for effectively reducing risk of occupational exposure to CFA.

OCCUPATIONAL EXPOSURE HAZARDS

Primary routes of occupational exposure to CFA include: accidental injection (Chapel and August 1975), conjunctival contact via splashing (Hanley, Bennett, and Artwohl 1995), and skin contact (Scott 2001). A number of industrial hygiene/epidemiology case studies have documented serious consequences in relation to acute and chronic occupational exposure.

Exposure to CFA via accidental injection typically produces serious and immediate health problems including: severe swelling, granulomatous inflammation, pain, lesions, abscesses, necrosis, and ulceration of tissues surrounding injection following the accidental injection of CFA (Chapel and August 1975). The related skin maladies and severe pain may persist for several years following the initial exposure incident (Chapel and August 1975) and is typically not responsive to antibiotic therapies (Broderson 1989, University Iowa IACUC, 2002). Limited studies have indicated possible benefits related to treatment of CFA-related granulomatous inflammations with corticosteroids/chloroquine (Shah et al 2001). Victims of accidental CFA injection also frequently experience influenza-like symptoms that may persist for several weeks (Chapel and August 1975). Chronic systemic effects including persistent fever, neurological abnormalities, tuberculoid granulomas, and arthritic symptoms have also been documented (Chapel and August 1975, Hanly, Bennett, and Artwohl 1995, Scheibner 2000). Cases of permanent organ damage, incurable autoimmune disease, and chronic disease potentiating live *Mycobacterium tuberculosis* following human injection of CFA has been reported (Matsumoto 2004, Scheibner 2000).

Eye and skin exposure to CFA via accidental splashing may lead to serious health complications. Occupational exposures involving accidental splashing of CFA into the unprotected eyes of workers during preparation of emulsions and other mixtures has reportedly produced symptoms including severe ocular irritation, scar tissue formation, and temporary/permanent vision impairment (Hanly, Bennett, and Artwohl 1995). Thorough studies involving toxicological effects of CFA contact with unprotected skin were not available during the preparation of this document. The limited available literature, however; indicates possible health and safety concerns including hypersensitivity and interference with Mantoux test (tuberculin test) following repeated skin exposure (discussed in detail below).

Case studies have also indicated that some individuals are inherently more sensitive to CFA and/or have a greater risk of developing acute hypersensitivity following repeated CFA exposure (Scott 2001). Such CFA hypersensitivity has been documented in individuals who have a prior history of testing positive to tuberculin tests (exhibit sensitivity to mycobacterial antigens) (Hanly, Bennett, Artwohl 1995). Hypersensitivity may also develop in relation to chronic skin exposure to even minute traces of CFA, as may be associated with mixing/preparing injections (Scott 2001). Studies have also indicated that any exposure to CFA may result in positive tuberculin tests (Chapel and

August 1975). Eliciting of such a “false positive” indication can result in unnecessary stress/medical treatment to affected individuals and in turn eliminates the use of the tuberculin test as a diagnostic screening tool thereby placing the individual and community at elevated risk.

SAFE WORK METHODS

The list of potential CFA-related health hazards identified above make it imperative that PIs conduct thorough risk assessments and prepare protocols which include standard operating procedures (SOPs) which identify appropriate administrative controls, personal protective equipment (PPE), work methods, engineering controls, and waste disposal procedures for eliminating or sufficiently reducing exposure threat to all staff involved in the affected research.

1. Administrative Controls

a. Prior to incorporating CFA into any protocol, consider using other, less problematic adjuvants. A list and description of other widely used adjuvants can be accessed at link below:

<http://research.uiowa.edu/animal/?get=adjuvant>

b. Management considerations for CFA and other potentially hazardous chemicals must be included in the laboratory [Chemical Hygiene Plan](#).

c. All IACUC protocols involving the use of CFA must register the agent with the IBC via the Appendix C for Chemical Hazards on the IACUC submission form.

d. Principal investigators will develop and implement standard operating procedures (SOPs) by which laboratory staff will prepare/administer CFA with minimal potential for exposure.

e. All tasks having potential for occupational CFA exposure (mixing of emulsions, dose preparation, administering of injections, etc.) will only be conducted by competent staff whom have received appropriate training (OSHA: “Worker Right to Know”) regarding the specific CFA-related health and safety risks, SOPs, and procedures to be

followed in event of an exposure incident.

f. All staff engaging in processes identified above are also required to complete applicable modules of the [VCU Laboratory Safety Training Modules](#).

2. Personal Protective Equipment: Staff involved with any tasks where potential for CFA exposure exists (mixing of emulsions, preparation of doses, injection of animals, handling of contaminated carcasses and/or bedding materials) must don the following PPE:

- a. Examination gloves.
- b. Safety glasses or safety goggles (ANSI Z-87 approved).
- c. Lab coat.
- d. Appropriate laboratory attire.

3. Work Methods

a. Needles used for CFA injection will be disposed of in approved sharps containers immediately following use.

b. Needles used for CFA injection should never be bent, sheared, or recapped. If recapping is absolutely necessary, a "[Needle Recapping Waiver](#)" must be submitted for IBC review/approval prior to proceeding.

c. Areas where CFA is prepared and/or administered should be cleaned immediately following each task completion utilizing a 5% to 10% bleach/water solution (prepare fresh stock as needed).

4. Engineering controls

a. In cases where the recommended level of PPE does not provide sufficient protection (e.g.: large splash potential) tasks should be conducted within a biological safety cabinet or chemical fume hood utilizing sash for added protection.

b. Syringes used for CFA injection must be safety engineered (self-sheathing syringes, luer-lock syringes, etc.).

c. Animals should be appropriately restrained and/or sedated prior to administering injections.

5. Waste Disposal

a. CFA-contaminated carcasses, bedding, and other nonsharps materials must be disposed of as [Regulated Medical Waste](#) (RMW).

b. All contaminated sharps waste materials must be placed in proper sharps container and disposed of as RMW.

LITERATURE CITED

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