CYCLOSPORIN A:
SAFE WORKING PRACTICES INFORMATION PAGE:

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PURPOSE

The purpose of this page is to provide the principal investigators with information regarding health threats, exposure routes, proper work methods, and provisions of suitable personal protective equipment for development of research protocols that effectively reduce the risk of occupational exposure to Cyclosporin A (CsA).

BACKGROUND

Cyclosporin A (CsA, CAS No. 59865-13-3) is a strong immunosuppressant agent (Laupacis et al., 1982; Wagner, 1983) used for the treatment of kidney, liver, heart, and other organ transplantation; rheumatoid arthritis; and psoriasis (Faulds et al., 1993). Cyclosporin A is a non-polar cyclic polypeptide consisting of 11 amino acids produced by multiple fungal species (Petcher et al., 1976; Krensky et al., 2006; Budavari et al., 1996). Cyclosporin A is a white to off-white crystalline solid that is slightly soluble in water and soluble in organic solvents (Budavari et al., 1996). Combustion or decomposition of CsA produces carbon monoxide, carbon dioxide, nitrogen oxides, hydrogen chloride gas, and phosgene (Sigma, 2000). Cyclosporin A indirectly elicits an immunosuppressive response via an interaction with immunophilin, a cytoplasmic protein. The CsA/immunophilin complex interacts with calcineurin to block phosphatase activity. The inhibited calcineurin-catalyzed dephosphorylation results in the failure of the T-lymphocyte to respond to specific antigenic stimulation (Schreiber and Crabtree, 1992). Metabolism of CsA occurs in the liver by CYP3A enzyme and to a lesser degree in the gastrointestinal tract and kidneys (Fahr, 1993). The metabolism of CsA yields approximately 18-25 metabolites that possess little biological activity and toxicity compared to the parent compound (Christian and Sewing, 1993; Ellenhorn et al., 1997). Long-term exposure to CsA has been linked to an increased risk of cancer (IARC, 1990), teratogenicity (HSDB, 2003; Novartis, 2003) and reproductive toxicity (Misro et al., 1999). The negative health effects associated with CsA present a significant health threat to laboratory staff, animal handlers, and others whose duties routinely place them at risk to accidental exposure. Due to this health and safety threat the Institutional Biosafety Committee (IBC) has classified CsA as a reportable hazardous chemical that must be reported on Institutional Animal Care and Use Committee (IACUC) protocols.

OCCUPATIONAL EXPOSURE HAZARDS

Primary routes of occupational exposure to CsA include: inhalation, accidental injection, and dermal absorption (NIOSH, 2004; NTP, 2005). Epidemiological data available for occupational exposure to CsA is limited. However, chronic effects in patients and laboratory animals treated with CsA are well documented. The available scientific literature indicates that chronic exposure to CsA could lead to a number of serious health effects.

1. Carcinogenicity: Cyclosporin A is "known" human carcinogen (IARC, 1990; NTP, 2005). It has been shown to produce cancer in both laboratory animals and humans. Common malignancies associated with long-term CsA exposure are lymphoma and skin cancer (Micromedex, 2001). Laboratory animals exposed to CsA experienced an increased incidence of lymphoma (IARC, 1990). Current scientific evidence suggests that CsA causes sister chromatid exchange in human lymphocyte cells *in vitro,* and unscheduled DNA synthesis and chromosomal aberration in the peripheral blood lymphocytes of kidney transplant patients treated in combination with prednisolone (IARC 1990). An increased incidence of cancer among patients undergoing immunosuppressive therapy has been documented following CsA use (IARC, 1990). Evidence has suggested that the risk of developing a malignancy during CsA treatment is likely the result of immunosupression and not genotoxicity (Ryffel, 1992; Novartis, 2003).

2. Genotoxicity: Cyclosporin A is non-genotoxic to humans (McClain et al., 2001). Cyclosporin A has not been found to be genotoxic in a variety of assays designed to test for mutagenic/genotoxic characteristics (Novartis, 2003).

3. Teratogenicity: The teratogenicity of CsA is not well documented. However, the limited amount of data available suggests that CsA is embryo and fetotoxic. Fetal mortality was observed in rats and rabbits when CsA was administered during pregnancy at 2-5 times the normal human dose (IARC, 1990; Novartis, 2003). A retrospective study of 116 pregnancies of women who received cyclosporin A during pregnancy showed that the only consistent pattern of abnormality was premature birth and low birth weight for gestational age. Several malformations and deaths were also reported during the study (Micromedex, 2001).

4. Reproductive Toxicity: Reproductive toxicities associated with CsA are primarily observed in males following long-term exposure. Cyclosporine A has induced dose dependent changes in reproductive organ weights and caused sterility at high doses in male rats (Handelsman et al., 1984). In addition, CsA has been shown to impair testicular functional and epididymal sperm maturation in laboratory animals (Seetalakshmi, et al., 1987). In humans, spermatozoa from renal transplant patients treated with CsA have shown decreased motility and viability resulting in decreased fertility (Bantle et al., 1985; Misro et al., 1999).

SAFE WORK METHODS

The list of potential CsA-related health hazards identified above necessitates the need for principal investigators to conduct thorough risk assessments and prepare protocols which include measures for minimizing staff exposure potential. To date, governmental regulatory agencies have not established exposure limits for CsA. In lieu of the availability of regulatory guidance, the prudent course for principal investigators to follow is to either eliminate or reduce exposure potential as much as feasible through implementation of the safe work methods listed below.

1. Administrative Controls.

a. Management considerations for CsA and other potentially hazardous chemicals must be included in the laboratory Chemical Hygiene Plan.

b. Protocols involving the *in vivo* use of CsA must include completion of IACUC Hazardous Chemical Information Page and approval through the Institutional Animal Care and Use Committee.

c. Principal investigators will develop and implement standard operating procedures (SOPs) by which laboratory staff will prepare/administer CsA with minimal exposure.

d. All tasks having potential for occupational CsA exposure (mixing of doses, dose preparation, administering of injections, etc.) will only be conducted by competent staff who have received appropriate training (OSHA: "Worker Right to Know") regarding the specific CsA-related health and safety risks, SOPs, and procedures to be followed in event of an exposure incident.

e. Laboratory personnel using CsA in any of the procedures noted above are also required to complete applicable modules of the VCU Laboratory Safety Training Modules.

f. Laboratory personnel must be instructed to use extreme caution when performing injections involving CsA since accidental needle stick presents an exposure threat.

g. Exposures involving CsA or any other acutely hazardous material should be reported to Employee Health as soon as possible.

2. Personal Protective Equipment. Cyclophosphamide exposure may often be attributable to the wearing of inadequate PPE. Staff involved in any tasks where potential for CsA exposure exists must don the following PPE:

a. Examination gloves: Use powder-free latex, nitrile, or rubber examination gloves which cover hands and wrists completely through overlapping sleeve of lab coat when working with CsA. Wearing of two sets of gloves ("double gloving") is advised whenever performing tasks involving CsA and other hazardous/antineoplastic drugs. Laboratory personnel should thoroughly wash hands with soap and water before and immediately upon removal of examination gloves.

b. Safety glasses or safety goggles (ANSI Z-87 approved) are considered the minimum appropriate level of eye protection. The IBC recommends donning of full-face shield when conducting tasks posing potential for any generation of aerosol or droplets.

c. Lab coats or disposable coveralls that provide complete coverage of skin not otherwise protected by PPE and/or attire. Laboratory personnel whose clothing has been contaminated by CsA should change into clean clothing promptly. Do not take contaminated work clothes home – contaminated clothing should be disposed of as regulated medical waste (RMW). .

d. Appropriate laboratory attire: laboratory personnel handling CsA should don attire which when worn in combination with lab coat and other PPE provides entire coverage of the body. Short pants/dresses and open-toed shoes are not appropriate laboratory attire.

e. If an aerosol exposure threat exists, all procedures should be conducted in an approved chemical fume hood whenever possible (see Engineering Controls below). If an approved chemical fume hood cannot be utilized, an appropriate air-purifying respirator must be utilized for all

procedures where exposure potential is present. A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use. Prior to instituting respiratory protection to personnel, the laboratory must participate in the university Respiratory Protection Program.

3. Work Practices:

a. Procedures with the potential for producing CsA aerosols should be conducted within an approved chemical fume hood whenever possible.

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

c. Needles used for CsA 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.

d. Bench paper utilized during preparation of CsA stock should be lined with an impervious backing to limit potential for contamination of work surfaces in the event of the occurrence of minor spills.

e. Areas where CsA is prepared and/or administered should be cleaned and decontaminated immediately following each task. Bench tops, BSC interiors, equipment, and laboratory surfaces with potential for CsA contamination should be routinely cleaned with a minimum of a 70% alcohol solution or other suitable deactivating agent: prepare fresh stock only as needed (LC Laboratories, 2006).

f. Do not eat, smoke, or drink where CsA is handled, processed, or stored, since exposure may occur via ingestion. Wash hands carefully before eating, drinking, applying cosmetics, smoking, or using the restroom.

4. Engineering Controls:

a. Use of chemical fume hood is recommended for all tasks with potential of aerosolizing CsA. In all cases where engineering controls alone do not sufficiently reduce exposure potential, provision of appropriate PPE for suitably minimizing hazard will be required.

b. Syringes used for CsA injection must be safety engineered (self-sheathing syringes, luer-lock syringes, etc.). Exceptions will be considered by the IBC on a case-by-case basis.

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

d. Laboratories and other spaces where handling of CsA occurs must be equipped with an eyewash station that meets American National Standards Institute (ANSI) and OSHA requirements.

5. Waste Disposal:

a. Cyclosporin A is a hazardous material, surplus stocks and other waste materials containing greater than trace contamination (> 3%) must be disposed of through the university hazardous waste disposal program. Trace amounts (<3% by weight) must be disposed of as Regulated Medical Waste (RMW) (NIOSH, 2004).

b. Cyclosporin A and its metabolites are principally excreted though the bile into the feces, with only about 6% excreted in urine. Only about 0.1% of CsA is excreted as unchanged drug (Krensky et al, 2006). The elimination half-life is generally biphasic and occurs approximately 5-18 hours following an intravenous injection (Fauld et al., 1993; Noble and Markham, 1995). The metabolism and potential risks associated with CsA use require that all potential contaminated carcasses, bedding, and other materials be disposed of as RMW through incineration.

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

6. Spills: Laboratory personnel must don appropriate PPE prior to attempting to manage any spill involving hazardous drugs/antineoplastic agents. University policy for addressing spills involving CsA is provided below:

a. Small spills (typically involving less than 5 mg of material) of CsA powder should be wet-wiped with cloth/gauze that is dampened with soapy water. Effected surfaces should be thoroughly wet-wiped three times over with at least a 70% alcohol solution (LC Laboratories, 2006)) – with clean damp cloth used for each wipe down. Following completion all cloth and other materials utilized during spill clean-up with potential for CsA contamination must be disposed of as RMW.

b. Small spills (typically involving less 5 ml of material) of liquid CsA should be covered/absorbed with absorbent material. Areas affected by liquid spills should be triple cleaned with soap and water following removal of absorbent paper.

c. For larger spills of CsA, contact the OEHS emergency line (828-9834) for assistance.

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