CYCLOPHOSPHAMIDE:

SAFE WORKING PRACTICES INFORMATION PAGE

Revised 10/30/09

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 Cyclophosphamide (CPH).

BACKGROUND

Cyclophosphamide (Cytoxan®, CPH, CAS No. 50-18-0) is an antineoplastic compound that is chemically related to nitrogen mustard. Cyclophosphamide is an odorless, fine white to off-white crystalline powder that is soluble in water and ethanol (NTP, 2005). Cyclophosphamide is used clinically to treat a wide range of cancers including malignant lymphomas, myeloma, leukemia, mycosis fungoides, neuroblastoma, adenocarcinoma, retinoblastoma, and breast carcinoma (Bristol-Myers Squibb Co, 2003). Other clinical uses for CPH include immunosuppressive therapy following organ transplants or as a treatment for autoimmune disorders such as rheumatoid arthritis, Wegener's granulomatosis, and nephritic syndrome in children (Chabner et al., 2001). Metabolism of CPH takes place in the liver and undergoes metabolic activation by cytochrome P450 isoenzyme 2B (Chabner et al., 2006). The major circulating metabolite of CPH, 4-Hydroxycyclophosphamide, is in equilibrium with its tautomer, aldophosphamide, which is spontaneously broken down to produce phosphoramide mustard and acrolein (Zhang et al., 2005). Phosphoramide mustard is responsible for anti-tumor effects, while acrolein is responsible for the hemorrhagic cystitis observed during CPH therapy (Chabner et al., 2006). Cyclophosphamide is a known alkylating agent with alkylating properties that result in nucleotide base mispairs and DNA/DNA or DNA/protein cross-linking that lead to major disruptions in nucleic acid function and the inhibition of DNA synthesis (Zhang et al., 2005). Cyclophosphamide-induced nucleic acid damage may lead to DNA mutations that result in cytotoxicity, carcinogenicity, teratogenecity, and reproductive toxicity following chronic exposure to CPH (NTP, 2005; Gilian and Charzinoff, 1983; Mirkes, 1985; Meirow et al., 2001). The negative health effects associated with CPH present a significant health and safety threat to laboratory staff, animal handlers, and other personnel who may be subject to accidental exposure. Due to this health and safety threat the Institutional Biosafety Committee (IBC) has classified CPH as a reportable hazardous chemical that must be registered on the Institutional Animal Care and Use Committee (IACUC) protocol Appendix C for Chemical Hazards.

OCCUPATIONAL EXPOSURE HAZARDS

Primary routes of occupational exposure to CPH include: inhalation, accidental injection, and dermal absorption (NIOSH, 2004; NTP, 2005). A limited number of studies have examined chronic health effects related to occupational exposure to CPH and have reported an increased incidence of cancer among health care workers (Sessink et al., 1993). However, chronic effects in patients treated with CPH are well documented. The available scientific literature indicates that chronic long-term exposure to CPH could lead to a number of serious health effects.

1. Carcinogenicity: Cyclophosphamide is a known alkylating agent that has been sufficiently studied in a variety of *in vivo* and *in vitro* assays (NTP, 2005). In host-mediated assays, CPH induces chromosomal aberrations, sister chromatid exchange, and gene conversions (IARC, 1987). Laboratory animals exposed to CPH by various routes of administration develop benign and malignant tumors of the bladder, breast, lungs, liver, and injection site (IARC, 1981). In addition, rats treated with CPH developed leukemia and lymphoma (IARC, 1981 and 1987). Several epidemiological studies have consistently found excesses of bladder cancer and leukemia among people treated with CPH for various medical conditions (IARC, 1981; Kinlen, 1985; Pedersen-Bjergaard et al., 1985; Greene et al., 1986; Haas et al., 1987). Cyclophosphamide is classified Group 1 by IARC (1981), as a "known" human carcinogen.

2. Cytotoxicity: The cytotoxic effects of CPH are generally considered to be the result of DNA crosslink formation through covalent bonding of highly reactive alkyl groups of the alkylating nitrogen mustards (Zhang et al 2005). The alkylation of the 7-nitrogen atom of guanine in DNA molecules takes place by phosphoramide mustard resulting from CPH activation (Pette et al., 1995). At alkaline or neutral pH, the nitrogen mustard is converted to chemically reactive carbonium ion through imonium ion. Carboinium ions react with the N7 of guanine residues in DNA to form a covalent linkage. The second arm in the phosphoramide mustard can react with a second guanine moiety in an opposite DNA stand or in the same stand to form crosslinks (Fleer and Brendal, 1983; Springer et al., 1998). Following crosslink formation, the cells will undergo apoptosis initiated by DNA damage and inhibition of DNA replication, modulation of cell cycle, and other anti-proliferative effects (Bhatia et al., 1995; Chien and Ashman, 1986; Crook et al., 1986; Masta et al., 1995; O'Connor et al., 1991).

3. Teratogenicity: Cyclophosphamide is clearly teratogenic in animals with similar mutations reported in multiple laboratory animal species (Gilani and Charzinoff, 1983; Mirkes, 1985). Cyclophosphamide teratogenicity is characterized by central nervous system, skeletal, and facial anomalies (Gilani and Charzinoff, 1983; Mirkes, 1985; Padmanabhan and Singh, 1984). In addition, CPH is a known human teratogen with a recognizable pattern of malformation known as Cyclophosphamide Embryopathy (Vaux et al., 2003). Human malformations include growth deficiencies (pre- and postnatal) and central nervous system, facial, and skeletal anomalies (Vaux et al., 2003). Like the carcinogenic effects of CPH, the teratogenic effects are mediated through alkylating intermediates, phosphoramide mustard and acrolein (Mirkes, 1985; Stahlmann et al., 1985).

4. **Reproductive Toxicity**: Cyclophosphamide is associated with reproductive toxicities in both males and females (Bristol-Myers Squibb, 2003; Wetzels, 2004). Both spermatogenesis and oogenesis are interrupted following treatment with CPH (Wetzels, 2004). Cyclophosphamide induced sterility is dependent upon dose, duration of exposure, and the state of gonadal function at the time of exposure (Bristol-Myers Squibb, 2003; Wetzels, 2004). In females, amenorrhea has been associated with CPH exposure due to decreased estrogen and increased gonadotropin secretions (Wetzels, 2004). Late prepubescent females have developed ovarian fibrosis with complete loss of germ cells after prolonged CPH treatment (Bristol-Myers Squibb, 2003). Males exposed to CPH may develop oligospermia or azoospermia associated with increased gonadotropin release (Bristol-Myers Squibb, 2003; Wetzels, 2004). Cyclophosphamide induced reproductive toxicities may be reversible (Bristol-Myers Squibb, 2003).

SAFE WORK METHODS

The list of potential CPH-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 CPH. 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 CPH and other potentially hazardous chemicals must be included in the laboratory Chemical Hygiene Plan.

b. Protocols involving the in vivo use of CPH 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 CPH with minimal exposure.

d. All tasks having potential for occupational CPH 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 CPH-related health and safety risks, SOPs, and procedures to be followed in event of an exposure incident.

e. Laboratory personnel using CPH in any of the procedures noted above are also required to complete applicable modules of the VCU Laboratory Safety Training Modules.
http://www.vcu.edu/oehs/chemical/training/trainingmodules.pdf

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

g. Exposures involving CPH 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 CPH 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 CPH. Wearing of two sets of gloves ("double gloving") is advised whenever performing tasks involving CPH 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 CPH 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 CPH 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 CPH aerosols should be conducted within an approved chemical fume hood whenever possible.

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

c. Needles used for CPH 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 CPH 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 CPH 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 CPH contamination should be routinely cleaned with bleach (20%) or other suitable deactivating agent: prepare fresh stock only as needed (Hansel et al., 1997).

f. Do not eat, smoke, or drink where CPH 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 CPH. 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 CPH 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 CPH occurs must be equipped with an eyewash station that meets American National Standards Institute (ANSI) and OSHA requirements.

5. Waste Disposal:

a. Cyclophosphamide is a RCRA-listed hazardous material, surplus stocks and other waste materials containing greater than trace contamination 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. The available scientific literature indicates that CPH and its metabolites are primarily excreted in urine and has an elimination half-life of 3 to 12 hours. It is eliminated primarily in the form of metabolites, but from 5 to 25% of the dose that is excreted in urine is unchanged drug. Several cytotoxic and noncytotoxic metabolites have been identified in both urine and plasma. The metabolism and potential risks associated with CPH use require that all potentially 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 CPH is provided below:

a. Small spills – powder: (typically involving less than 5 mg of material) of CPH 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 a 20% bleach solution (Hansel et al., 1997) – with clean damp cloth used for each wipe down. Following completion all cloth and other materials utilized during spill clean-up with potential for CPH contamination must be disposed of as RMW.

b. Small spills – liquid: (typically involving less 5 ml of material) of liquid CPH 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 CPH, contact the OEHS emergency line (828-9834) for assistance.

References

Bhatia, U., Danishefsky, K., Traganos, F., Darzynkiewicz, Z. (1995). Induction of apoptosis and cell cycle-specific change in expression of p53 in normal lymphocytes and MOLT-4 leukemic cells by nitrogen mustard. Clin. Cancer. Res. 1:873–880.

Bristol-Myers Squibb Co. Cytoxan ® Package insert. Bristol-Myers Squibb Company. Princeton NJ. (2003)

Chabner, B.A., D.P. Ryand, Pax-Ares L, Garcis-Carbonero and Calaresi, P. (2001) Antineoplastic Agents. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th ed. J.G. Harnam and L.E. Libmirde, eds NewYork, NY: McGraw Hill. 1389-1459

Chabner, B.A.et al. (2006) Antineoplastic Agents. In Goodman and Gilman's The Pharmacological Basis of Therapeutics, 11th ed. J Brunton LL, Lazo JS, and Parker KL, eds NewYork, NY: McGraw Hill. 1322-1328, 1694

Chien, M. M., Ashman, R. F. (1986). The effects of nitrogen mustards on the proliferative and Ig synthetic response of human peripheral blood lymphocytes. Immunopharmacology 12:155–166.

Crook, T. R., Souhami, R. L., McLean, A. E. (1986). Cytotoxicity, DNA cross-linking, and single strand breaks induced by activated cyclophosphamide and acrolein in human leukemia cells. Cancer Res 46:5029–5034.

Fleer, R., Brendel, M. (1982). Toxicity, interstrand cross-links and DNA fragmentation induced by 'activated' cyclophosphamide in yeast: comparative studies on 4-hydroperoxy-cyclophosphamide, its monofunctional analogon, acrolein, phosphoramide mustard, and nor-nitrogen mustard. Chem. Biol. Interact. 39:1–15.

Gilian SH and Chatzinoff M (1983) Embryopathic effects of Cyclophosphamide. Environ Res 31:296-301

Greene, M.H., Harris, E.L., Gershenson, D.M., Malkasian, G.D., Jr, Melton, L.J., III, Dembo, A.J., Bennett, J.M., Moloney, W.C. & Boice, J.D., Jr (1986) Melphalan may be a more potent leukemogen than cyclophosphamide. Ann. int. Med., 105, 360-367

Hansel S, Castegnaro M, Sportouch MH, De Meo M, Milhavet JC, Laget M and Dumenil G. Chemical degradation of wastes of antineoplastic agents: cyclophosphamide, ifosfamide and melphalan. Int Arch Occup Environ Health. 1997; 69:109-114.

Haas, J.F., Kittelmann, B., Mehnert, W.H., Staneczek, W., Mohner, M., Kaldor, J.M. & Day, N.E. (1987) Risk of leukaemia in ovarian tumour and breast cancer patients following treatment by cyclophosphamide. Br. J. Cancer, 55, 213-218

IARC (1981) Some Antineoplastic and Immunosuppressive Agents. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Vol 26. Lyon, France: International Agency for Research of Cancer. pp 411

IARC (1987) Overall Evaluation of Carcinogenicity. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, Supplement 7. Lyon, France: International Agency for Research of Cancer. pp 440

Kinlen, L.J. (1985) Incidence of cancer in rheumatoid arthritis and other disorders after immunosuppressive treatment. Am. J. Med., 78 (Suppl. 1A), 44-49

Masta, A., Gray, P. J., Phillips, D. R. (1995). Nitrogen mustard inhibits transcription and translation in a cell free system. Nucleic. Acids Res. 23:3508–3515.

Meirow D, Epstein M, Lewis H, Negent D, and Gosden RG (2001) Administration of Cyclophosphamide at different stages of follicular maturation in mice: effects on reproductive performance and feta malformations. Human Reproduction 16:632-637

Mirkes PE (1985) Cyclophosphamide teratogenesis: a review. Tertat Carcin Mutagen 5:75-88

NIOSH Alert (2004) Preventing Occupational Exposures to Antineoplastic and Other Hazardous Drugs in Health Care Settings. National Institute of Occupational Safety and Health. NIOSH-Publications Disseminations: Cincinnati, OH

National Toxicology Program. Cyclophosphamide CAS No. 50-18-0 National Institute of Environmental Health Sciences. 11th Ed Report on Carcin. (2005)

O'Connor, P. M., Wassermann, K., Sarang, M., Magrath, I., Bohr, V. A., Kohn, K. W. (1991). Relationship between DNA cross-links, cell cycle, and apoptosis in Burkitt's lymphoma cell lines differing in sensitivity to nitrogen mustard. Cancer Res 51:6550–6557.

Padmanabhan R, Singh S (1984) Congenital anomalies of the ear resulting from cyclophosphamide treatment in the rat. Acta Anat 119:217-223

Pedersen-Bjergaard, J., Ersbøll, J., Sørensen, H.M., Keiding, N., Larsen, S.O., Philip, P., Larsen, M.S., Schultz, H. & Nissen, N.I. (1985) Risk of acute nonlymphocytic leukemia and preleukemia in patients treated with cyclophosphamide for non-Hodgkin's lymphomas. Comparison with results obtained in patients treated for Hodgkin's disease and ovarian carcinoma with other alkylating agents. Ann. Int. Med., 103, 195-200

Pette M, Gold P, Pette DF, Hartung HP, and Toyka KV (1995) Mafosphamide induces DNA fragmentation and apoptosis in human T-lymphocytes. A possible mechanism of its immunosuppressive action. Immunopharmacology 30:59-69

Sessink PJM, Van der Kerkhof MCA, Anzion RBM, Noordhoek J, Bos RP (1993). Environmental contamination and assessment of exposure to antineoplastic agents by determination of cyclophosphamide in urine of exposed pharmacy technicians: is skin absorption an important exposure route? Arch Environ Health 49(3):165–169.

Springer, J. B., Colvin, M. E., Colvin, O. M., Ludeman, S. M. (1998). Isophosphoramide mustard and its mechanism of bisalkylation. J. Org. Chem. 63:7218–7222.

Stahlmann R, Bluth U, Neubert D (1985) Effects of cyclophosphamide metabolite acrolein in mammalian limb bud cultures. Arch Toxicol 57:163-167

Vaux KK, Kahole NCO, Jones KL (2003) Cyclophosphamide, Methotrezate, and Cytarabine Embropathy: Is Apoptosis the Common Pathway? Birth Defects Research 67:403-408

Wetzels JFM (2004) Cyclophosphamide-induced gonadal toxicity: a treatment dilemma in patients with lupus nephritis? The Netherlands J of Med. 62:347-352

Zhang J, Tian Q, Yung S, Chuen S, Zhou S, Duan W, and Zhu Y. (2005) Metabolism and transport of oxazaphosphorines and the clinical implications. Drug Metabolism Reviews. 37:611-703