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DEVELOPMENT AND VALIDATION OF A METHOD FOR ACCURATE COLLECTION AND ANALYSIS OF SELECT ANTINEOPLASTIC CONTAMINANTS, BOTH IN AIR AND ON SURFACES, AND IMPLICATIONS FOR EXPOSURE ASSESSMENT

by

RODNEY R. LARSON

A DISSERTATION

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ABSTRACT OF DISSERTATION
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Title Development and Validation of a Method for Accurate Collection and Analysis of Select Antineoplastic Contaminants, Both in Air and on Surfaces, and Implications for Exposure Assessment

To conduct exposure assessment of antineoplastic contaminants in pharmacy and clinical use areas, an analytical method along with surface and air monitoring methods was developed and validated. Five antineoplastic agents were selected for this method based on their frequency of use and potential risk to human health. The agents selected were cyclophosphamide, ifosfamide, 5-fluorouracil, doxorubicin-HCl, and paclitaxel. Cyclophosphamide was the agent of most interest because it is listed by the International Agency for Research on Cancer (IARC) as a known human carcinogen. An analytical method developed was able to simultaneously analyze for all five agents of interest. For the surface monitoring method, Millipore #42, 55-mm filter circles were used for surfaces sample collection, and samples were desorbed in the same solvent blend determined to be optimal for use in wiping. The minimum concentration of detection for these agents was typically 2.0 ng/mm^2 or less for each of the five agents. The method for air monitoring was developed using a solid sorbent. Previous air monitoring had been done by use of TEFLON or glass fiber filters but with very questionable results. Information was provided at an international conference in Sweden that cyclophosphamide may actually sub-

limate or otherwise evaporate from high efficiency particulate air (HEPA) filters, which would indicate use of filters for monitoring may provide inaccurate results. Therefore, various solid sorbents were reviewed and tested for monitoring antineoplastics in air. Anasorb 708 (methyl acrylate polymer) tubes were found to be most acceptable for collection and desorbing of the all five agents of interest. The lowest concentration for detection in air of these agents was typically 0.5 $\mu g/m^3$ or less for each of the agents of interest when monitoring at 2.0 L/minute for 20 hours or more. The surface and air monitoring methods were tested at a hospital oncology pharmacy area and at a cancer treatment clinic and were found able to detect contaminants such as cyclophosphamide on surfaces at concentrations less than 1.0 ng/cm^2 and in the air at concentrations less than 1.0 $\mu g/m^3$. The ability to detect these agents at concentrations less than 1.0 $\mu g/m^3$ for assuring controls used to protect health care workers was adequate.

DEDICATION

I dedicate this dissertation to my beloved wife, Janice M. Larson. She sacrificed a lot, both socially and economically, so that I could complete this work. I shall be eternally grateful to her for her love and support.

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I am indebted to the other members of my committee: Mr. Max Richard, who originally provided the idea to evaluate health risks to oncology health care workers; Dr. Alfred A. Bartolucci in Biostatistics; and Dr. Deodutta Roy in Environmental Health Sciences. I believe I was most fortunate to have access to and counseling from these members of my research advisory committee. In addition, Mr. Bob Collum, a health control officer in the UAB Occupational Health and Safety Office and a registered pharmacist, was very helpful in assisting me with interfacing with the hospital and clinic administration and in obtaining the agents of interest for use in method development. My sincere thanks to all who assisted me in this study.

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A NEW MONITORING METHOD USING SOLID SORBENT MEDIA FOR EVALUATION OF AIRBORNE CYCLOPHOSPHAMIDE AND OTHER ANTINEOPLASTIC AGENTS

LIST OF ABBREVIATIONS

ACN	Acetonitrile
AGCIH	American conference of governmental industrial hygienists
ASHP	American Society of Hospital Pharmacists
ASTM	American Society of Testing and Materials
AU	Absorbance units
AU/V	Absorbance units per Volt
AUFS	Area under full scale
BSC	Biological safety cabinet
CA	Chromosome aberrations
CI-MS	Chemical Ionization-Mass Spectroscopy
C.V.	Coefficient of variation
CHO	Chinese hamster ovary
CP	Cyclophosphamide
DNA	Deoxyribonucleic Acid
DOP	Di(2-ethylhexyl) phthalate
GC	Gas Chromatograph
GC-MS	Gas Chromatography-Mass Spectroscopy
GC-MS-MS	Gas Chromatography-Mass Spectroscopy-Mass Spectroscopy or Gas Chromatography-Tandem Mass Spectroscopy
HCS	Hazard Communication Standard (OSHA)

LIST OF ABBREVIATIONS (Continued)

HEPA	High efficiency particulate air, or High extraction particle arresting
HPLC	High performance liquid chromatograph
IARC	International Agency for Research on Cancer
i.d.	Interior diameter
ID	Inside Dimensions
MDL	Minimum detection limit
MeOH	Methanol
MN	Micronuclei
NIOSH	National Institute for Occupational Safety and Health
NSC	National Study Commission (on Cytotoxic Exposures)
OSHA	Occupational Safety and Health Administration
PFP	Phenomenex Curosil Pentafluorophenyl
PPE	Personal protective equipment
PTFE	Polytetrafluorethylene (or TEFLON)
PVC	Polyvinyl chloride
QC	Quality control
RNA	Ribonucleic Acid
SCE	Sister-chromatid exchange
UAB	University of Alabama at Birmingham
UV	Ultraviolet light
UV/VIS	Ultraviolet-Visible Spectrophotometer (or visible based analysis)

INTRODUCTION

Initial plans were for conducting risk assessment studies on oncology nurses and pharmacists working with anticancer (chemotherapy) agents within hospitals and cancer treatment clinics. Exposure information to determine risk was to be gathered by use of existing monitoring methods being used to monitor these agents. However, during the preparation of the research proposal for conducting the risk assessment, new information was discovered which called the validity of existing monitoring methods into question. Therefore, the research plan was modified to the development of accurate and sensitive analytical and collection methods for the agents of most interest.

Interest in the occupational exposures to anticancer agents has increased because many of these agents have been found to be mutagenic and carcinogenic in experimental systems.[1-6] The International Agency for Research on Cancer (IARC) currently lists nine antineoplastic agents and one combination chemotherapy regimen as human carcinogens (group 1), which includes cyclophosphamide), six as probable human carcinogens (group 2A), five as possible human carcinogens (group 2B), and several as unclassifiable as to carcinogenicity to humans (group 3, which includes isophosphamide). In addition, second malignancies are known to be associated with several specific therapeutic treatments.

It is estimated that there are currently more than 50 cytotoxic drugs commercially available for treating cancer patients.[7] Thousands of health care workers are involved in

preparing and administering cytotoxic drugs, which include many of the antineoplastic drugs, and the number is expected to increase in proportion to the number of patients with cancer.[8] The American Cancer Society estimates that 1.268 million new cases of cancer (excluding carcinoma *in situ* and basal and squamous cell skin cancer) would be diagnosed in the United States in 2001.[9] Anticancer agents thus represent a class of occupational carcinogens and mutagens, and the handling should involve no unnecessary exposure.[10,11]

To assure unnecessary exposures to these chemotherapy agents do not occur requires availability and proper use of appropriate exposure controls. Such controls include personal protective equipment (PPE), engineering controls, and good handling procedures.[10] To verify that once in place these controls are effective in controlling exposure requires availability of accurate methods for detecting and quantifying exposures to these agents at levels low enough to be meaningful.[12] Recent information on existing monitoring methods, especially those used for monitoring certain antineoplastics in air, indicate they may not be sufficiently accurate.[13]

At a seminar in Sweden in July 1999, information was presented which indicated concern for the effectiveness of high extraction particle arresting (also known as high efficiency particulate air [HEPA]) filters for control of antineoplastic agents in biological safety cabinets (BSC).[14] It appeared that during use of cyclophosphamide, an agent with a very low vapor pressure at room temperature, that it volatilized and escaped through the HEPA filter. Molecules of vaporized cyclophosphamide (in gas form) would be <0.003 μm in diameter, which is much smaller than the pore size of HEPA filters (0.3 μm); thus, the gaseous particles would not be retained by the filters in the BSCs and would pass into

the work area. If this were true in the workplace, health care workers could be exposed to vapors of cyclophosphamide (and possibly other drugs) by inhaling contaminated air.

This information would also indicate that the existing methods using filters to monitor for cyclophosphamide would not be effective. In reviewing the results reported from most monitoring for air contaminants by the filter method, even when using long sample periods (e.g., 40+ hours) at high sample volumes, concentrations of cyclophosphamide at very low concentrations or at concentrations below levels of detection were found.[15-18] This initially was considered a favorable indication of good contaminant control; however, based on the recent information, cyclophosphamide and possibly other antineoplastics are either not being captured or not being retained after capture by the sample filter.

Besides the loss of the agent off or through the filter, it was further determined that the cyclophosphamide which escaped from the BSC would sublimate onto essentially all solid surfaces having common ventilation to the oncology pharmacy area(s). This vaporization and sublimation of cyclophosphamide could then result in widespread surface contamination, providing several opportunities for skin contact by all individuals working in such areas.

This information on surface contamination was supported by recent studies conducted in 1999 by Connor et al. in six cancer treatment centers in North America.[19] Contamination by antineoplastics was found in higher than expected concentrations in several areas besides in the oncology pharmacy areas.

Based on this information, it appeared necessary to develop an effective monitoring method for antineoplastic agents, at least for those agents believed to be inaccurately

detected by current methods. It is very important to have accurate methods for determining contaminant concentrations in air and on surfaces to more effectively implement and use the control technology for handling antineoplastic agents. Accurate methods for measuring exposure will ensure the highest possible level of protection for health care workers.

Therefore, this research focused on the activities listed: (1) development of an accurate analytical method for detection and quantification of the antineoplastic agents of interest and for the detection at the lowest attainable concentrations present; (2) development of a surface monitoring method acceptable for detection of the agents of interest, including the development of an effective solution for removal of all agents of interest for analytical purposes; and (3) Development of an air monitoring method acceptable for detection and quantification of the agents of interest.

A large amount of chemotherapy involving antineoplastic drugs is conducted in hospital treatment oncology pharmacies and in clinic or outpatient therapy. For this reason, the research focused on those antineoplastic drugs most commonly prepared or prepared in high concentrations at either or both of these types of facilities. A field validation of the monitoring methods developed from this research was also carried out in those facilities (see Conclusions).

A preliminary list of the antineoplastic drugs in use was obtained from these two types of facilities earlier. From this list, the drugs used most frequently and/or used in relatively high concentrations were identified (Appendix). This group of drugs was further limited to those that have similar potential to produce secondary cancer effects such as leukemia. This was believed important because exposure to them in combination at

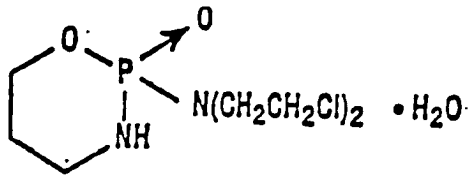
low doses during a work shift and over a long period of time (e.g., several years) may have the potential for synergistic effects that would increase the risk from such exposures.

From the information gathered above, the primary focus of this method development research was put on cyclophosphamide. This was because of it being both frequently used and a known human carcinogen and because of a desire to use it as a marker of exposure. The four other agents of interest identified for inclusion in the development of analytical and monitoring methods besides cyclophosphamide were ifosfamide, 5-fluorouracil, doxorubicin-HCl, and paclitaxel (Taxol). These five agents generally have unique chemical structures (Figure 1), as well as varied chemical properties, making the task of developing a single method acceptable for all five agents a challenge.[20]

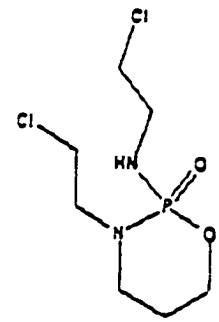
Background on chemotherapy agents

To begin with, it is important to understand the agents included in this study are part of chemotherapy treatment of cancer patients. Chemotherapy refers to the prevention or treatment of disease by chemical agents[21] or the use of any of a group of drugs whose main effect is either to kill or slow the reproduction of rapidly multiplying cells.[22,23] Although not all of these chemotherapy agents have been studied by their manufacturers for risk of secondary cancer to patients being treated for cancer, essentially all manufacturers of these products caution physicians and pharmacists to consider that they probably are at risk.[24,25] The highest secondary cancer risk to patients appears to be leukemia.[26] It is this carcinogenic potential of these chemotherapy drugs being handled

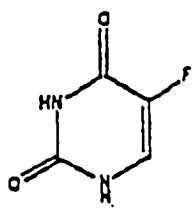
Figure 1. Chemical structures of antineoplastics to be studied



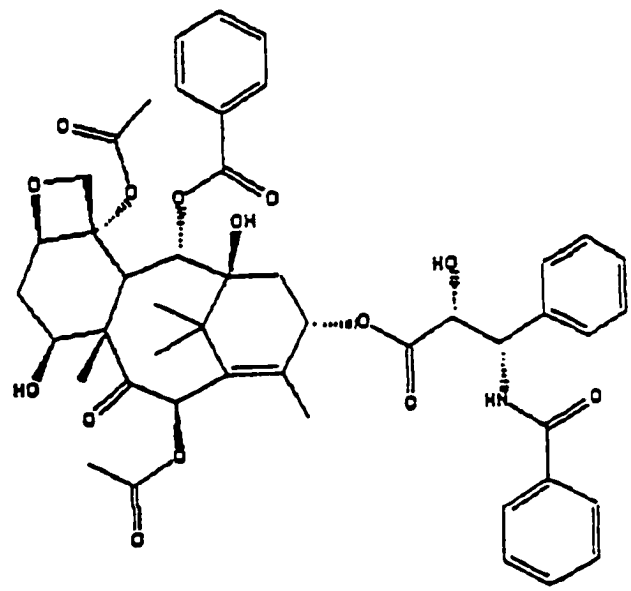
CYCLOPHOSPHAMIDE
$C_7H_{15}Cl_2N_2O_2P \cdot H_2O$



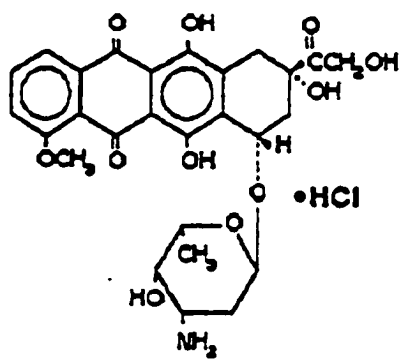
IFOSFAMIDE (Isophosphamide)
$C_7H_{15}Cl_2N_2O_2P$



5-FLOUROURACIL
$C_4H_3FN_2O_2$



PACLITAXEL (Taxol)
$C_{47}H_{51}NO_{14}$



DOXORUBICIN HYDROCHLORIDE
$C_{27}H_{29}NO_{11} \cdot HCl$

by oncology pharmacists and nurses that has raised interest in evaluating their exposures and associated risk.[27] Of relevance to this study is the sharp increase in use of antineoplastic agents in recent years because of (1) identification of new cancers and new cancer cell types with metastasis to different areas, and (2) effectiveness of antineoplastic treatment protocols as a primary and adjunctive medical treatment for cancer.

Various toxic effects from mucous membrane, eye and skin irritation, dizziness, nausea, and headache to chromosomal aberrations, fetal loss, congenital malformation, and other effects such as bone marrow suppression, liver damage, and cutaneous toxicity have been reported. It should be noted that most antineoplastic agents are supplied as powders in vials (some may be liquid solutions) that require reconstitution or dilution before administration by intravenous or intramuscular injection. Hospital personnel may be exposed by both inhalation and dermal contact. Powder particles may become airborne and liquid droplets may become aerosolized during preparation or administration or through accidental spills. These drugs may also contaminate clothing, skin, and hospital surfaces as a result of leaks, splashes, or spills. A nursing association has reported episodes of extravasation associated with the administration of these agents by office assistants, aides, and others that have received minimal training.[28]

History. Early in 1942, Yale University and the Office of Scientific Research and Development entered into a contract to investigate chemical warfare agents.[29] The group involved in this research identified the unique and fascinating properties of the nitrogen mustards. Certain basic observations were made early in the course of the study of these compounds. It was immediately appreciated that, in addition to a local vesicant ac-

tion, the nitrogen mustards were cytotoxic following absorption. Death of experimental animals was the result of systemic effects even after topical application to the skin. The susceptible tissues were those with renewal call populations, primarily lymphoid tissue, bone marrow, and the epithelium of the gastrointestinal tract. A major effort of the Yale group was devoted to a study of the relationship of the stage of transformation of nitrogen mustard to its distribution, pharmacodynamic actions, and toxicity. After research involving rabbits, it was learned that normal lymphoid tissue had a remarkable sensitivity to the cytotoxic action of the nitrogen mustards. This led to the question being raised: Could one destroy a tumor with this group of cytotoxic agents before destroying the host? Subsequently, studies were conducted on mice that showed the ability of the nitrogen mustards to significantly reduce certain cancer tumors. This information resulted in the first test on a human in December 1942 on a patient at Lindskog in the terminal stages of lymphosarcoma. After 4 days of treatment, some improvement was noted, with significant improvement on day 10 when treatment was stopped. These tests and their results lead to development of the nitrogen mustards cyclophosphamide and ifosfamide used today.

Since that first use of nitrogen mustard on the patient with lymphosarcoma in late 1942, many alkylating agents have been synthesized and tested for antitumor activity. All these compounds are capable of reacting in a manner such that an alkyl group or a substituted alkyl group becomes covalently linked to cellular constituents. After the first successful experiments with alkylating agents, many other types of anticancer drugs have been designed, such as antimetabolites, antibiotics, hormones, and mitotic inhibitors. In 1985, Sorsa et al.[30] indicated approximately 30 anticancer drugs were in clinical use and

many others were under development. Several new anticancer drugs have subsequently been developed since 1985, adding to the potential for risk to health care workers.

All commonly used mustards, such as nitrogen mustard and cyclophosphamide, have sufficient evidence for carcinogenicity both in humans and in animals, and are listed as group 1 carcinogens by the IARC.[4,5] In humans, the chemotherapeutic use of cyclophosphamide includes a variety of secondary tumors such as leukemias and bladder tumors. The drugs belonging to the class of nitrogen mustards are also mutagenic in various test systems and cause chromosomal aberrations in treated patients.

Antineoplastic exposure hazards

Antineoplastic drugs include chemically unrelated classes of agents. They are capable of inhibiting tumor growth by disrupting cell division and killing actively growing cells.[31] These same mechanisms, however, may be carcinogenic, mutagenic, and/or teratogenic in and of themselves.[32] Laboratory evidence of the biologic activity of some of these agents has triggered concern about the potential long-term health risks to persons handling these drugs.[18] Some studies have failed to identify a relationship between measures of mutagenicity and exposure to antineoplastic agent.[33-37] There is, however, a growing body of evidence that indicates otherwise.[14,38-45]

Hansen and Olsen[46] have reported significant increases in the incidences of non-melanoma skin cancer and non-Hodgkin's lymphoma (standardized incidence ratios of 1.5 [95% confidence interval, 1.1-2.1] and 3.7 [95% confidence interval, 1.2-8.9], respectively) in Danish female pharmacy technicians. In industrial settings, worker exposure to known carcinogens is closely regulated. However, the health care sector has been rela-

tively exempt from regulatory control, with Occupational Safety and Health Administration (OSHA) issuing only guidelines.[47,48] The recommendations published by the European Union are nonobligatory but applicable to every workplace where there is a risk of exposure to carcinogens. These recommendations include three levels of protection: (1) reduce or replace a carcinogen as far as technically possible; (2) if reduction or replacement is not possible, manufacture or use the carcinogen in a closed system; and (3) limit quantities, limit the number of exposed workers, use engineering controls to minimize release into the working environment, use protective equipment, and perform other protection-related activities.

Many adverse effects of antineoplastic drug therapy have been reported. These include occurrences of "second malignancies" and hematopoietic effects,[49-51] impaired reproductive function,[52] and immunosuppression.[53-56] These reports and laboratory evidence of the mutagenic activity of antineoplastics have triggered concern about possible health risks to hospital personnel handling these drugs. Information on concerns specific to individual antineoplastics included in this study is indicated below.

Although other antineoplastic agents are planned for evaluation, cyclophosphamide is one of the most commonly prepared and administered antineoplastic drugs, and, because it has been indicated as being a class 1A carcinogen by IARC, it is the focus of this study.

Cyclophosphamide. Cyclophosphamide or (2-[bis(2-chloroethyl) amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate) and ifosfamide are alkylating agents. The alkylating agents are frequently implicated as responsible for adverse

verse consequences, notably in patients who receive adjuvant or therapeutic doses. This class of drugs substitutes alkyl groups for hydrogen in certain organic compounds. The alkylation of nucleic acids in deoxyribonucleic acid (DNA) is the critical action of most of these compounds, resulting in breaks of the molecule, interfering with DNA replication and the transcription ribonucleic acid (RNA).[38]

Cyclophosphamide is an antineoplastic drug that is biotransformed principally in the hepatic cytochrome P-450 system (CYP2B group of P-450 isoenzymes) to form active alkylating metabolites.[57-59] Cyclophosphamide, like many other antineoplastics, is classified by the IARC as an animal and human carcinogen, mutagen, and teratogen.[1-3]

Today cyclophosphamide is one of the most extensively used chemotherapeutic agents in the treatment of cancer. The carcinogenic and mutagenic characteristics of cyclophosphamide are well documented, thus enabling the application of genotoxicological methods, in addition to chemical ones, in the environmental monitoring of potential exposure situations.[60-62] It should also be noted that the alkylating agents are known to be teratogenic.[38,63] As indicated above, sampling for cyclophosphamide was chosen for this study to be a marker agent for antineoplastic exposure.[18] A combination of several factors led to the choice of cyclophosphamide to be used as a primary marker for exposure. First, cyclophosphamide is the drug of choice for the treatment of many types of cancer. For example, cyclophosphamide is used for treatment of malignancies such as malignant lymphomas, Hodgkin's disease, multiple myeloma, a variety of leukemias, adenocarcinoma of the ovary, and breast cancer.[57,61] Second, it is an essential component of many effective drug combinations such as cyclophosphamide combined with doxorubicin and

5-fluorouracil for therapy after breast cancer surgery.[59] Thus, cyclophosphamide is used extensively.[18]

According to hospital pharmacists and recommendations by the manufacturer, cyclophosphamide is used in relative high doses, frequently in the hundreds of milligrams of cyclophosphamide per patient per day, or, in the case of bone marrow or peripheral stem cell rescue, patients may be given doses of 5 to 7 g/m^2 over a 3-day period.[57,59]

Ifosfamide. Ifosfamide (isophosphamide) is a structural isomer of cyclophosphamide with one of the chloroethyl side chains residing on the ring.[64] It was synthesized several years after cyclophosphamide, and both remain clinically the most useful alkylating agents to date.[65] There are substantial differences in their spectrum of antitumor activity and toxicity,[66] and these differences may be in part due to differences in metabolism and stability of several of their metabolites. The difference in metabolism and resulting metabolites is believed to be due to the difference in P-450 systems involved in the metabolic process. Ifosfamide is hydroxylated by the CYP3A system (P-450 isoenzymes)[67] and may account for the somewhat different pattern of antitumor activity, different pharmacokinetic profiles, and the interpatient variability in toxicity.[59]

Like cyclophosphamide, ifosfamide[64] is used in relatively high doses (1-6 g/day). It is usually used in combination with certain other approved antineoplastic agents and is indicated for the third-line chemotherapy of germ cell testicular cancer. It should not be used for patients with severely depressed bone marrow function or patients who have demonstrated a previous hypersensitivity to it. Urotoxic side effects, especially hemorrhagic cystitis, have been frequently associated with the use of ifosfamide. Ifosfamide

has been shown to be carcinogenic in rats, with female rats showing a significant incidence of leiomyosarcomas (a benign tumor) and mammary fibroadenomas. The mutagenic potential of ifosfamide has been documented in bacterial systems *in vitro* and mammalian cells *in vivo*. *In vivo*, ifosfamide has induced mutagenic effects in mice and had induced a significant increase in dominant lethal mutations in male mice as well as recessive sex-linked lethal mutations in *Drosophila* (fly).[67]

5-Fluorouracil. 5-Fluorouracil is a fluorinated pyrimidine.[68] It is an antimetabolite, thought to block the methylation reaction of deoxyuridylic acid to thymidylic acid, interfering with the synthesis of deoxyribonucleic acid (DNA) and to a lesser extent, inhibiting the formation of ribonucleic acid (RNA). Because DNA and RNA are essential for cell division and growth, the effect of 5-fluorouracil may be to create a thymine deficiency, which results in cell death. Effects of DNA and RNA deprivation are most marked in those cells that grow more rapidly and that take up 5-fluorouracil at a more rapid rate. This is usually the case with cancer cells but would also indicate reason for exposure concern for pregnant women due to rapid fetal cell development. For this reason, 5-Fluorouracil falls in pregnancy category D, Teratogenic effects risk. That is, it may cause fetal harm when administered to a pregnant woman.

It is not clear if 5-fluorouracil is carcinogenic due to lack of acceptable animal studies. Studies have been conducted on male rats with oral doses of up to 3 mg/rat, 5 days a week for 52 weeks, and a 6-month observation period after dosing stopped. Assuming up to 800 mg per day for an adult human male, assuming 75 kg average weight, and a male rat average weight of 0.5 kg, the highest animal test dose (3 mg/day) would be

equivalent to 450 mg per day for a human. This is much less than the manufacturer's maximum recommended daily dose of 800 mg. However, 5-fluorouracil has been shown to mutagenic to several strains of *Salmonella typhimurium* and to *Saccharomyces cerevisiae*.[69]

Doxorubicin-HCl. Doxorubicin-HCl is a cytotoxic anthracycline antibiotic isolated from cultures of the fungus *Streptomyces peucetius* var. *caesius*.[70] It damages DNA by intercalation of the anthracycline portion, metal ion chelation, or generation of free radicals.[71] The most common uses for doxorubicin in cancer therapy is for various types of leukemia, breast cancer, Ewing's sarcoma, Hodgkin's disease, lung cancer (small cell), non-Hodgkin's lymphoma, ovarian cancer, and soft tissue sarcoma. Common two-medication regimens receiving attention today are combinations of doxorubicin and dacarbazine used in various treatment protocols for sarcomas and Hodgkin's disease and doxorubicin and vincristine mixtures used to treat hematologic cancers and Kaposi's sarcoma. (Stewart et al., 1997) Doxorubicin is also used, but less frequently, for bladder cancer, endometrial cancer, lung cancer (non small cell), neuroblastoma, retinoblastoma, testicular cancer, thyroid cancer, and a variety of other cancers. Doxorubicin has been shown to have mutagenic and carcinogenic properties in experimental models. Its effects on fertility have not been established.

Paclitaxel. Paclitaxel (Taxol) is a diterpene amide.[72] It is an antimicrotubule agent and was first isolated from the bark of the Pacific Yew (*Taxus brevifolia*).[73] Mechanism of action is that taxol disrupts the equilibrium between free tubulin and

microtubules by shifting it in the direction of assembly. It promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions.

Taxol binds extensively to plasma proteins. It undergoes significant P-450 mediated hepatic metabolism to hydroxylated metabolites, and this is believed to be the major mechanism of elimination. Therapeutically, it has significant importance for ovarian cancer, breast cancer, carcinoma of the lungs, and head and neck carcinoma, with fairly high response rates. The major toxicity is bone marrow depression with neutropenia the common dose limiting toxicity. Because hypersensitivity reactions are common, it is recommended that all patients be pretreated with corticosteroids, diphenhydramine, and H_2 antagonists. However, fatal reactions to Taxol have occurred in some patients despite premedication. The carcinogenic potential of Taxol has not been studied. It was not found to be mutagenic in the Ames test, but Taxol can cause fetal harm when administered to a pregnant woman.

Potential for exposure

To identify and conduct the appropriate type of monitoring for antineoplastic drugs are important to evaluate exposure risk. This first requires the determination of the potential for exposure, starting with an evaluation of the type(s) of facilities and the activities carried out in the facility that may provide an opportunity for exposure. An understanding of the routes of exposure and under what circumstances such exposure may occur is also very important.

It is believed that the major routes of occupational exposure to cytotoxic chemotherapy drugs are from inhalation of the aerosolized drug and from absorption by direct skin contact with surface contamination.[74] There are numerous opportunities for such exposures within a manufacturing plant or a health care facility (e.g., pharmacy, hospital, cancer center). Obviously, the potential for exposure will vary dependent on the type of facility involved (i.e., manufacturing versus health care facility), engineering controls present, the use of PPE, and the training and awareness of those individuals handling the hazardous agents.

Potential for exposure to antineoplastics in manufacturing. The potential for exposure in the manufacturing area is very different from that in the hospital care centers (hospitals, cancer centers, etc.). To emphasize this difference, the following is an example of the manufacturing of cyclophosphamide. This example indicates a typical method of manufacturing and the activities that may lead to exposure of individuals involved in that activity.

Exposure potential during manufacturing of cyclophosphamide. The manufacturing process of cyclophosphamide was studied by Pyy et al.[75] They found the process took place in two reaction steps; the first step produced CP II (phosphoroxydichloride mustard) from CP I (*nor*-nitrogen mustard) and phosphoroxytrichloride. In the second phase, unpurified cyclophosphamide was produced from a reaction of CP II with 3 aminopropanol and triethylamine. In the manufacturing area, the reactors are loaded manually by the process workers from upstairs in the production hall (860 m^3). The ven-

tilation in the production hall of the plant had an average air exchange rate of 8.3 times/hour, and additional local ventilation was installed in the process machinery.

The finishing operation of cyclophosphamide was performed in a separate hall, where unpurified cyclophosphamide is dried in a drying drum, loaded into fibrous 20-kg barrels and transported into another plant where cyclophosphamide drug production took place. Cyclophosphamide was packed into vials as powder, but cyclophosphamide tablets are also produced. Cyclophosphamide powder was prepared in the aseptic department using laminar flow hoods and packed by machines into 10-kg vials. The workers wore purified air helmets (airstream). Dust measurements for the manufacturing area were collected on 3 days successively and were repeated 18 month later. Sampling times varied from 20 minutes to 18 hours. Samples were collected on 37-mm glass-fiber filters, at 20 L/minute (0.5-30 m^3) for fixed point sampling and 2 L/minute for personal sampling. Total sample volume per sample ranged from 100-1000 liters. Results of air monitoring in the manufacturing areas/operations are indicated in Table 1.

Dust measurements in the drug production areas of the plant were performed on 2 days successively and were repeated 9 month later. Sampling times varied from 30 minutes to 7 hours. Monitoring results for this area are also shown in Table 1. Two genotoxicity assays, following the United Kingdom Environmental Mutagenicity Society standard procedures[76,77] were conducted on the air samples collected in the manufacturing area. First, the filter halves of ambient air samples were dissolved in sterile water under sonication. Second, the samples were tested using the Ames/plate incorporation assay (± S9) using *S. typhimurium* TA 1535 as the indicator strain and the sister-chromatid

Table 1.

Cyclophosphamide in Air Concentrations from Manufacturing and Drug Production Areas

Job Area	Type of Sample	No. of Samples	Range ($\mu g/m^3$)
		Manufacturing Areas	
Process	Fixed Point	19	0.1 - 0.4
	Personal	9	0.6
Centrifuge	Fixed Point	7	0.1 - 12.0
	No Personal	0	
Drying Drum	Fixed Point	4	10.0 - 810.0
	Personal	2	97.0 - 190.0
		Drug Production Areas	
Tableting Department			
Weighing	Fixed Point	4	2.0 - 13.0
	No Personal	0	
Mass Preparation	Fixed Point	4	190.0 - 480.0
	Personal	1	160.0
Tableting	Fixed Point	9	4.0 - 6.0
	Personal	3	6.0 - 32.0
Support Works	Fixed Point	6	None Detected
	No Personal	0	
Aseptic Department			
Powder Preparation	Fixed Point	3	1.0 - 2.0
	Personal	2	32.0
Powder Filling	Fixed Point	5	1.0 - 7.0
	Personal	6	2.0 - 17.0
Checking	Fixed Point	2	0.4 - 0.5
	No Personal	0	

From Pyy et al., 1988.[25]

exchange (SCE) assay in Chinese hamster ovary (CHO) cells (±S9 mix) using 4-h pulse treatment.[60]

Evidence of health concern from the manufacturing of cyclophosphamide. The genotoxicity testing was conducted on the process intermediates in the cyclophosphamide manufacturing process. *Nor*-nitrogen mustard (CP I) and phosphoroxydichloride mustard (CP II), and other major raw material ingredients in the production process (diethanolamine, triethylamine, and 3-aminopropanol) were tested. Diethanolamine, triethylamine, and 3-aminopropanol showed no genotoxic activity in the SCE/CHO cell system, either with or without the exogenous metabolic system. However, both process intermediates, CP I and CP II, were found to be highly active both in the Ames/plate incorporation assay and in the SCE/CHO cell assay. Further, a significant dose-related response was obtained both with and without the exogenous metabolic system.

Exposure potential in health care facilities (hospitals, cancer centers)

There are a number of activities carried out by both oncology pharmacists and nurses (and, in some cases, physicians) that can lead to their being exposed to antineoplastic (potentially cytotoxic) agents. These activities, because of possible contamination to air or surfaces in the work area, may also result in exposure to individuals not directly involved in the handling of the these potentially hazardous agents.

For example, antineoplastic agents are prepared and used in many hospitals, clinics, and pharmacies.[78] Antineoplastic drugs are usually dissolved in a liquid for intrave-

nous administration to the patient. The greatest hazard to health care personnel is during the preparation of these solutions.[79-83] Another important source of exposure is the administration of solubilized drugs and antineoplastic drugs in tablet form to the patient. The person preparing or administering drugs may be exposed through direct skin contact or through inhalation of an aerosolized drug.[84-88] Direct skin contact may occur after spills or from spraying and spattering during preparation. Use of contaminated work clothing or penetration of protective gloves may also cause contact with skin.[89]

Examples of manipulations that can cause splattering, spraying, and aerosolization include (1) insertion and withdrawal of needles from drug vials, (2) drug transfer using syringes and needles or filter straws, (3) breaking open ampules, and (4) expulsion of air from a drug-filled syringe. New evidence further indicates potential for airborne contamination and subsequent exposure due to volatilization of the antineoplastics off the HEPA filters on BSCs that return air to the work area.

Skin contact and subsequent contamination can occur (1) during the opening of the ampules, (2) from spill and splatters, and (3) from contact with surfaces contaminated by aerosols (counter tops, tables, etc.), or (4) from cleaning the interior of BSCs (usually recommended to be done weekly).

Further, it appears that antineoplastics volatilize and/or sublimate off BSC HEPA filters will eventually condense or return to the solid state on essentially any and all solid surfaces in the area(s) where the BSC exhaust air is released. This would include surfaces such as the exterior of cabinets, bench/table tops, carts, trays, floors and walls, and even clothing.

Evidence of exposure. Despite awareness of these possibilities for exposure, the true extent of exposure to the drugs among health care professionals is not known.[74] However, as a result of exposure potential to health care workers, reports of occupational exposure to antineoplastic agents and the effects of these agents on such workers do continue to appear in the literature. In a 1984 survey[84] of current handling practices of anticancer drugs in five major U.S. hospitals, it was estimated that a total of 23,000 clinically used therapeutic doses were handled in a year. Of these, 14% were alkylating agents, 22% were antibiotics, and 64% were antimetabolites.[30]

This study also identified that the potential for exposure can vary between those health care workers working in oncology departments of hospitals and those in outpatient departments.[90] The preparation by pharmacy personnel may be relatively similar, as is much of the administration by nurses, but there is a difference in exposure among these groups of workers based on a large extent to their individual practices for handling the antineoplastic agents during preparation and administration. One other difference is due to the patients in the oncology department needing on-going care, which includes the nurse being exposed during washing the patient and removal and cleaning of urinals and chamber pots and in handling of clothing and bed linen of patients.[61] This study will not be evaluating the exposures in these types of situations. As for details on exposure to pharmacy workers, as indicated above, many of the antineoplastic drugs must be reconstituted, transferred from one container to another, or manipulated before administration to the patients. Even when care is taken, an opportunity for absorption through inhalation or direct skin contact can occur.[32,49,83,87] Assessment of actual exposure is a technically difficult procedure. Some reasons for the difficulty are as follows: (1) most users

handle a variety of drugs during the workday, (2) the quantity of materials mixed or administered during any single procedure is relatively small, and (3) generally the mixing and administration schedules vary significantly from day to day.

Of interest to this proposal, a study conducted by Sessink et al. (1992)[16] on environmental contamination and excretion of cyclophosphamide in exposed workers monitored both the air concentration and surface contamination of cyclophosphamide in the pharmacy area of a cancer therapy center. The drugs were prepared in laminar airflow safety hoods, and the pharmacy technicians wore protective clothing, nurse's caps, masks, and gloves. Two air samples were taken, each on separate days during preparation of cyclophosphamide and 5-fluorouracil, using 50-mm diameter, 0.45-μm pore size cellulose filters. On day 1, 6.73 m^3 air was sampled, and on day 2, 5.55 m^3 air was sampled. No cyclophosphamide or 5-fluorouracil was detected.

Contamination of the working tray in the hood was detected once after preparation. Contamination of the floor with 5-fluorouracil was found at all locations on at least one of the two days. Cyclophosphamide was not detected in any of the wipe samples taken from the floor. Gloves were found to be contaminated with cyclophosphamide and 5-fluorouracil, and 5-fluorouracil was detected not only during preparation of this drug but also during preparation of other drugs. Further, some of the packings of the drugs were contaminated on the outside. One vial of cyclophosphamide was contaminated with 0.06 μg cyclophosphamide on the outer surface. Contamination with 5-fluorouracil was detected on the outside of the packings of three prepared products (two 5-fluorouracil cassettes, one infusion bag) at 1.4, 3.1, and 4.0 μg, respectively. Cyclophosphamide was detected at 2.1 μg on the outside of a cyclophosphamide infusion bag.

Another method successfully used to demonstrate exposure relative to surface contamination is a "simulation" technique involving use of a saline-soluble fluorescent dye for evaluating the potential contamination by cytotoxic drugs of the work area. Simulation of maneuvers identical to those used during actual drug handling techniques, including mixing and administration, was conducted. A black light was used to visualize drugs that have been aerosolized or splattered and have contaminated either a sampling frame or the protective clothing. Contamination was observed on the gloves and sleeves of the participants; the protective aprons were splattered with the fluorescent dye, particularly in the upper chest area; and surface contamination was observed on the workstation, the vials, and the syringe(s). Because this contamination was observed during the mixing procedure, it is logical to assume the aerosol or splatter also went in the direction of the user.[74]

Evidence of reproductive risk concerns to workers in health care facilities

Work by Valanis et al.[90-91] illustrated some of the recent adverse acute and reproductive effects related to occupational exposure. Findings from this study, as well as data published previously on exposure to antineoplastic agents, emphasize the need for all health care personnel who prepare or administer these agents and personnel working in adjacent or surrounding areas to strictly adhere to published guidelines. Health care personnel who handle these drugs must become more aware of the potential for contamination and should improve their techniques and containment procedures to reduce exposure. Other workers in the area need to be aware of the potential for exposure to this ever-present contamination.

Substantial levels of contamination from three antineoplastic agents were detected on a variety of surfaces in pharmacy drug preparation areas and a drug administration area in six cancer treatment centers in Canada and the United States.[19] In 1985, a study on abortion and malformations among nurses working in selected departments of general hospitals in Finland was conducted by Hemminki et al.[92] This covered a period from 1973 to 1979 and included case nurses who had had a spontaneous abortion (n=219) or a malformed child (n=46) study reported. Information on exposures during the first trimester of pregnancy was obtained from the head nurses of the hospitals. No significant increase in risk of spontaneous abortion or malformation was observed after exposure to anesthetic gases, sterilizing gases and soaps, or X-rays. In the later phase of the study, it was extended to include the department of extensive cancer treatment (e.g., oncology departments). Results indicated handling of cytostatic drugs (nurses selected based on handling cytostatic drugs at least once per week) did not affect the frequency of spontaneous abortion, but there was a strong association with malformations in offspring (odds ratio was 3.4). This study, done in general hospitals and not where extensive chemotherapy is carried out, did indicate cytostatic drugs do cause a strong reproductive risk.

According to new evidence from the Netherlands, nurses, pharmacists, and other health care professionals who regularly prepare and/or administer anticancer drugs are up to five times more likely to give birth to babies with congenital defects and 40% more likely to experience spontaneous abortions. These and other alarming findings were unveiled at an international conference on cytotoxic exposure risks and hazards held June 17, 1999, in Stockholm, Sweden. At the day-long conference, researchers from Germany, Holland, Sweden, and the United States presented overwhelming data to show that

health care workers are at much greater risk than previously thought from their exposure to anticancer drugs.[14]

Cyclophosphamide concerns related to exposures in health care facilities. Occupational handling of anticancer agents in hospitals has been shown to be associated with manifestations of ill health, such as an increase in spontaneous abortions and congenital malformations.[92,93] Results with biological monitoring and cytogenetic surveillance methods have given both positive and negative results in occupational exposure situations. The discrepancies may depend on differences in potential exposure (e.g., handling practices, extent and quantities handled) or methodological limitations.

However, positive findings have been obtained from geographically and methodologically very different studies. These findings include an increase in point-mutation frequencies in the lymphocytes of nurses and pharmacists in New Zealand,[94] increases in chromosomal aberrations and SCE in laboratory assistants in Czechoslovakia,[95] and oncology nurses in Finland[96-98] and in Norway.[51]

Urine samples have revealed chemically measurable amounts of cyclophosphamide in some samples of nurses handling anticancer agents in Canada[81] and in the Netherlands.[99] These data, in connection with ambient-air measurements showing cyclophosphamide to be present in quantities up to 0.37 $\mu g/m^3$ in the dilution room,[15] argue for special consideration to be taken in the prevention of potential occupational exposure.[39]

A cancer risk assessment study conducted by Sessink et al.[61] evaluated exposure risk to health care workers based on both toxicology (animal) studies and on epidemiology studies involving tumor incidence data of patients. The risk based on the animal data

indicated a rate of 120 to 600 cases per million of urinary bladder tumors and a rate of 95 to 475 per million for leukemias. The results for exposed workers based on the epidemiology data indicated 20 to 100 per million leukemias in women. This study did indicate a dose-response relationship between exposure to cyclophosphamide and the risk of cancer, and it did show that the workers have a higher risk for cancer due to the handling of cyclophosphamide and/or other antineoplastic agents in spite of the protective measures that had been taken.

Concerns potentially associated with use of engineering controls

In the early 1980s, a study by Anderson et al.[34,52] compared the use of a BSCs with the practice of using a horizontal-laminar-airflow hood as a means of reducing worker exposure to antineoplastic agents. These authors concluded that the BSC provided greater protection from exposure.

A study by Pyy et al.[75] involved collection of air samples from three types of hoods during dilution and dispensing of cyclophosphamide for injection and infusion vials. In a hospital pharmacy, within a vertical laminar flow hood equipped with a HEPA filter, 8500 mg of cyclophosphamide was prepared during a 90-minute period. All samples taken from inside and outside the hood were below the analytical limit of detection (0.05 $\mu g/m^3$). When an intentional spill of cyclophosphamide was arranged, a small amount of cyclophosphamide (10 $\mu g/m^2$) was found under the bottom cover paper in a wipe sample. Air samples from two smaller hoods used less intensively for the preparation of cytostatic drugs were negative for cyclophosphamide during normal dissolution practices of 2000 mg and 4000 mg of cyclophosphamide. When the HEPA filter material

used for 1 year in the laminar flow hood in the hospital was analyzed, cyclophosphamide was detected in all three samples, with an average of 510 $\mu g/m^2$ of filter material. Pyy et al. did note that aerosol spills may occur even with the very experienced handling practices of the hospital pharmacy.

Other investigators evaluated the use of BSCs and other means of personal protection, finding a reduction in exposure as measured by urine mutagenicity and other biological monitoring techniques.[19,100] However, Sessink et al.,[16,61,101,102] Ensslin et al.,[103,104] and others,[99,105] using more sensitive analytical procedures than those used in earlier studies, documented measurable levels of antineoplastic agents in urine samples collected from pharmacists, technicians, and nurses who were using BSCs and gloves as the minimum level of protection. This recent information indicates reliance on BSCs to provide total protection from exposure to antineoplastic agents may be misguided and may provide a false sense of security.

Additionally, Schmidt recently gave a presentation at a seminar on exposure to antineoplastic drugs in Stockholm, Sweden,[14] indicating possible concern associated with BSCs that return air to the work area through HEPA filters. Schmidt discussed vapor pressures of cytotoxic drugs and the implications for occupational safety. His interest in the topic had first started when he had been approached, because of his experience in aerosols, for advice on how to dispose of spent filters from BSCs. The cabinets had been used for preparation of cytotoxic drugs, and so it was expected that the filters would be heavily contaminated and unsuitable for incineration at normal temperatures. He had analyzed the filters and found no traces of cytotoxic drugs. Schmidt had concluded that the drugs had "vanished like the vapor of moth balls."

Considering the situation as it pertained to vertical laminar flow cabinets, Schmidt said that large particles (diameter >0.5 μm) moved with the airflow (and with gravity) to the bottom of the cabinet, whereas gas molecules (diameter >0.003 μm) floated with the stream. They were subject to Brownian movement, moved down concentration gradients, and could even come out into the room (through the hand opening of a BSC).[14]

Turning to HEPA filters, Schmidt explained that they were tested by measuring the number and distribution (by diameter) of particles, both upstream and downstream of the HEPA filter. In his department, equipment had been designed to test the efficiency of HEPA filters with respect to cytotoxic aerosols. Experiments had compared the effect of drug solution applied to the filter with that of drug aerosol. Ten percent of the drug in solution passed the filter, whereas 45% of aerosolized drug passed the filter. These results did not fit with the theory (that all aerosolized drug would be trapped by the filter) and stimulated the search for another explanation.[14]

"We had to consider the possibility of evaporation," said Schmidt. Further experiments had shown that small aerosol particles of, for example, 5-fluorouracil and cyclophosphamide, evaporated in a few seconds. Thus, he suggested, small aerosol particles were deposited on the HEPA filter but then evaporated and passed through the filter (which was not designed to absorb vapors).[14]

Another experiment had shown that cyclophosphamide powder held in a closed beaker for 28 days at room temperature (25 °C) evaporated and sublimed onto a glass fiber filter and a chromatographic strip suspended above it in significant quantities. Schmidt's team had concluded that any process that involved the generation of cytotoxic particles was likely to result in contamination of the surrounding area. They had then

tested the theory by measuring surface contamination with cytotoxic drugs in a cytotoxic drug preparation area in a hospital pharmacy. The room contained a BSC, which returned the filtered air to the room. Although the room was not in use at the time and had been cleaned on the previous day, cyclophosphamide vapor was detected (via air filter samples) at the exhaust outlet at a level of 130 $\mu g/m^3$, and most surfaces in the room were contaminated with cyclophosphamide. In particular, a wall of glass brick appeared to be heavily contaminated, and this was thought to be because the drug was adsorbed on to the surface of the glass, explained Schmidt.[14]

Exposure risks from vaporization/surface contamination

Scientists in Germany have now shown that several commonly used anti-cancer drugs vaporize at room temperature and pass through the air in clinics and rooms where these injections are prepared. Unless special precautions are taken, staff that work in these areas are likely to inhale small quantities of these drugs, even under stringent safe-handling standards and with the precautions of laminar air flow cabinets and high extraction particle arresting (HEPA) filters.

Professor Graham Sewell (University of Bath) asked, at the seminar in Sweden, [14] if isolators should be fitted with carbon filters to remove cytotoxic drugs, as many of them returned filtered air to the room in which they were situated. Schmidt replied to Sewell that he thought that the charcoal filters would quickly become saturated with isopropyl alcohol and would probably need to be regenerated every 12 to 24 hours in order to remain effective.

Further, unanticipated high levels of surface contamination, both inside and outside preparation rooms, were documented in a study of six cancer centers in the United States and Canada, confirming additional risks even for those health care workers not directly involved in preparation or administration.[19]

Also, Sessink et al.[16,101,106] detected cyclophosphamide in the urine of workers who were not directly involved in drug preparation. In one study by Sessink et al. on evaluation of occupational exposure to antineoplastic agents at several departments in a hospital, floors of different antineoplastic handling areas were tested for surface contamination. In the antineoplastic administration room of one outpatient department, the floors were found to be contaminated with cyclophosphamide at the beginning of the working day, although no cyclophosphamide was detected at the end of the previous working day. The room had been cleaned in the meantime; therefore, it was suggested that the rooms were not cleaned efficiently and that the cleaning process may have spread the contamination. Based on the information from Dr. Schmidt, it appears possible that the contamination detected at the beginning of the day was a result of cyclophosphamide being emitted from a hood filter back into the room in the interim period between testing.

All these findings, coupled with the knowledge that protective equipment is not universally employed in many settings, increase the concern for health care workers who are directly or indirectly involved in the preparation, administration, and disposal of antineoplastic agents and who are at risk of exposure through environmental contamination.[79,107-111]

Air contaminant monitoring methods

Historically, researchers have assumed that antineoplastics contaminate air as solid or liquid (solubilized agent) aerosols as a result of handling practices indicated above. It was also assumed since they are not visible that these aerosols would be in relatively small concentrations and predominantly in the 1.0 to 10 μm range.[112]

Use of filters for air contaminant monitoring. Based on these assumptions, the most frequent method for air monitoring has been to use either fibrous or porous membrane filters. These are the most important types of filters for aerosol sampling. Fibrous filters consist of a mat of fine fibers arranged so that most are perpendicular to the direction of airflow. These filters are predominantly air, having porosities from 70 to greater than 99%. The most common types are cellulose fibers, glass fibers, and plastic fibers. The air velocity through high-efficiency (i.e., HEPA) filters is usually quite low, on the order of 10 cm/second (600 cm/minute or ~20 ft/minute).[113]

A common misconception is that aerosol filters work like microscopic sieves in which only particles smaller than the holes can get through. This assumption may be appropriate for the liquid filtration of solid particles but is not correct for how aerosol filtration works. Particle removal in a fibrous filter occurs by collision and attachment to the surface of the fibers. Porous membrane filters have a different kind of structure with less porosity, 50-90%. The gas flowing through the filter follows an irregular path through the complex pore structure. Particles are lost from the gas stream as they deposit on the structural elements that form the pores. Membrane filters have high efficiency and a greater pressure drop than other types of filters. The high collection efficiency of porous

membrane filters extends to aerosol particles much smaller than the manufacturer's stated pore size, which is based on liquid filtration. Porous membrane filters may be made from cellulose esters, sintered metals, polyvinyl chloride, Teflon, or other plastics. The common filter used in monitoring for antineoplastics in air has been either glass fiber or Teflon (PTFE or Poly-tetra-fluor-ethylene), either 37-mm or 50-mm diameter, and pore sizes of 0.45 or 0.5 μm.

If these assumptions for aerosol (particle) size were correct, the methodology would be very effective. There is a face velocity that gives minimum efficiency for a given particle size and filter. As velocity is increased, collection by diffusion is reduced and collection by impaction is increased. The velocity for minimum efficiency increases with decreasing particle size. The particle size that gives the minimum efficiency is about 0.2 μm. This is considered to be an in-between size that is too large for diffusion to be effective and too small for impaction or interception to be effective.

Because these competing mechanisms operate in different size ranges, all filters have a particle size that gives minimum efficiency, usually in the size range 0.05 to 5.0 μm. The collection efficiency of high-efficiency (i.e., HEPA) filters uses 0.3 μm DOP (di(2-ethylhexyl) phthalate) particles on the assumption that this size is near the minimum efficiency point and efficiency will be greater for all other sizes.

This information would indicate the existing use of filters for monitoring antineoplastics to be quite effective, which it probably is in the initial collection phase. But, very few air-monitoring samples have been able to indicate the presence of antineoplastics, even when the samples are collected over several days and significant evidence of surface contamination is determined present in the same areas. These observations, along with

those provided by Schmidt, would indicate the probability of sublimation occurring with drugs such as cyclophosphamide and 5-fluorouracil. Thus, there is very likely a rapid loss of these agents off the filter after initial collection.

Use of solid sorbents. Cyclophosphamide is a polar material, requiring a sorbent with both adsorption and desorption characteristics that are favorable for such compounds.[114] However, because of the interest in simultaneous monitoring of other antineoplastic agents, some of which are non polar, it will also be necessary to try to identify a sample media that has characteristics acceptable for both types of materials. In reviewing characteristics related to specific retention and breakthrough volumes for a variety of solute and adsorbent combinations, three sorbents were selected to be candidates for screening. These were Amberlite XAD-8 (now known as Anasorb 708), Porapak R, and activated carbon. The characteristics of these sorbents are indicated in Table 2.

The initial selection of sorbent(s) is focused on monitoring of cyclophosphamide and not combinations, since cyclophosphamide is being evaluated as a marker of antineoplastic contamination. Anasorb 708 and activated carbon have the highest enthalpy (ΔH) values for several organic vapors. The Anasorb 708 indicated it was likely to have high ΔH values for cyclophosphamide, based on ΔH values identified by Stanetzek et al.[115] for cyclohexylamine, a polar compound with high ΔH values available.

Activated carbon adsorbs polar compounds so strongly that a ΔH determination was only feasible for compounds such as acetone. ΔH values for nonpolar compounds, if determinable, are similar to ones obtained for Amberlite XAD-4 (dispersive interactions).

The higher the retention volume and the adsorption enthalpy, the stronger are the solute-surface interactions.

Table 2.
Properties of Candidate Sorbents for Cyclophosphamide Monitoring

Sorbent Name	Amberlite XAD-8 (Anasorb 708)	Porapak R	Activated Carbon
Specific surface area (m^2/g)	140	450-600	1000
Average pore diameter (Å)	250	76	Not Obtainable
Monomer composition	Methacrylic acid polymer	N-vinyl pyrrolidine polymer	Carbon (Coconut charcoal)
Adsorption enthalpy (ΔH) (re, cyclohexylamine)	70.5 kJ/mol	55 kJ/mol	No Values
Retention (V_g) & breakthrough volumes (V_D) & specific breakthrough volume (re, cyclohexylamine)	560 V_g(V_D) 310 (L/g)	330 V_g 330 (L/g)	No Values Decomposition Possible
Mesh Size	Not Available	50/80	Not Available

Stanetzek et al., 1996[115]

Note: V_g = Retention Volume (Retention time of solute molecule (t_R or minutes/gram) multiplied by the mobile phase flow rate (F or L/minute) or L/g).

V_D = Breakthrough Volume (Breakthrough time of solute molecule, which is when it is first detectable at the column outlet (t_D or minutes/g). multiplied by the mobile phase flow rate (F or L/minute) or L/g)

L/g = Specific Breakthrough Volume.

Stanetzek et al.[115] found Anasorb 708 has an efficient retention ability for the polar substances and may be most effective for cyclophosphamide, which is a polar com-

pound with a melting point of about 45 °C. According to Stanetzek, the most unspecific sampling materials are Porapak R and activated carbon, although activated carbon is well known as a sorbent for adsorption of highly volatile nonpolar compounds. It is noted that retention ability can be reduced if humidity is present. How much breakthrough is shortened depends on the adsorbents as well as on the pollutant. Field validation of the method(s) developed for sampling cyclophosphamide and other antineoplastics will be conducted to assist in providing some information on the impact from humidity and/or from the presence of other agents that may also be collected on the sorbent(s) chosen.

Protection from exposure

Pharmacists, nurses, physicians, and other health care workers may be exposed to potentially significant workplace levels of hazardous drugs, which include the cytotoxic drugs during preparation, administration, or disposal.[48] Monitoring results for cytotoxic drugs has detected measurable levels of both air and surface contamination. This was especially true when exhaust BSCs were not used for preparation.

Surveys of U.S. cancer centers and oncology clinics in the early 1980s revealed wide variation in work practices, equipment, or training for personnel preparing cytotoxic drugs.[15,117] This lack of standardization resulted in a high prevalence of potential occupational exposure to cytotoxic drugs. One survey found 40% of hospital pharmacists reported a skin exposure to cytotoxic drugs at least once a month, and only 28% had medical surveillance programs in their workplaces.[79] Nurses, particularly those in outpatient settings, were found to be even less well protected than pharmacists.[109] Such findings emphasized the lack of protection for all personnel who were at risk of exposure to haz-

ardous drugs.[48] Information from these and other surveys led OSHA and other organizations and individuals to develop guidelines on use of equipment and work practices to control exposures.[118-121]

Handling guidelines: government and other organizations. Section 5(a)(1) of the Occupational Health and Safety Act of 1970 requires an employer to provide a workplace "free from recognized hazards that are causing or are likely to cause death or serious physical harm to his employees." Under this General Duty clause, if an employer (e.g., a hospital pharmacy) is out of compliance with recognized industry standards of good practice, that employer can be cited for violating the act (OSHA Law, 1980). Additionally, some of the agents are covered under the Hazard Communication Standard (HCS) (29 C.F.R. Part 1910.1200). (OSHA, 1989, as amended Feb. 9, 1994)

OSHA has published two documents to provide information that could be used in compliance situations. In 1986, the first guidance document, titled "Work-Practice Guidelines for Personnel Dealing with Cytotoxic (Antineoplastic) Drugs," was published by the OSHA.[116,117,122] OSHA described this document as an "informational guidance document."

In 1996, OSHA published a second guidance document on this subject titled "Controlling Occupational Exposure to Hazardous Drugs." This informational document was an expansion from the earlier (1986) guidelines to cover hazardous drugs in addition to the cytotoxic drugs.[48] The American Society of Health-System Pharmacists also published a Technical Assistance Bulletin on Handling Cytotoxic and Hazardous Drugs, which they indicated is still current.[123]

Personal protective equipment. Besides concern for exposure to hazardous drugs through inhalation, exposure is possible via skin contact.[81,124] Several studies have examined handling practices and found many instances where adherence to published guidelines (OSHA, American Society of Hospital Pharmacists [ASHP]) to be minimally adequate or poor.[123, 125-129] The most common recommended safety precautions include use of the proper type of BSC and personal protective equipment such as gloves and gowns. A recommendation in the guidelines is for change-out of the gloves at least once every hour or immediately if they are torn, punctured, or contaminated with a spill.[116,123]

A number of reports on permeability testing of glove materials for protection from cytotoxic drugs have appeared in the literature.[125-129] Earlier reports indicated surgical latex gloves provide the best protection against a number of chemotherapy drugs. Further research indicated that the thickness of the gloves used in handling hazardous drugs is more important than the type of material, since all materials tested were found to be permeable to some hazardous drugs.[123,125,126] The best results were seen with latex gloves. Thicker, longer latex gloves that cover the gown cuff were recommended for use with hazardous drugs, and gloves with minimal or no powder are preferred since the powder may absorb contamination.[123,130,131] It was noted in the studies that there was great variability in permeability within and between glove lots. Therefore, double gloving is recommended if it does not interfere with an individual's technique.

Subsequent to the above information, the availability of newer types and thicker glove materials have appeared on the market.[125] Tests were conducted on several of these newer materials by Connor et al., using standard American Society for Testing and Materials (ASTM) procedure.[132] Agents relative to this study that were also evaluated for

glove material permeability by Connor et al. include cyclophosphamide, 5-fluorouracil, and doxorubicin, and all were in an aqueous (water) solution when tested. It is assumed that, because of similarity in chemical structure, the results observed for cyclophosphamide would be the same for ifosfamide. Paclitaxel (Taxol) was the only agent of interest not included in this study by Connor et al.

The testing was carried out over a 2 hour test period. Results for the drugs of interest indicated no breakthrough at the end of 2 hours for the following gloves (Table 3). One of three samples of a glove product named Chemo + Plus was found to be permeable to 5-fluorouracil at 1 hour. Because of the thickness of the Chemo + Plus glove (0.46 mm) it was believed this single failure was due to a flaw (e.g., a break) in the integrity of the material rather than permeation. The Kendall Curity was found acceptable for the agents of interest in this study but was found unacceptable for carmustine, which is another antineoplastic.

Table 3.
Gloves Tested for Permeability

Product Name	Manufacturer	Description/ Material	Thickness (mm)
Digit-Safety Halo-Kote	Digitcare Corp.	Nonsterile seamless latex gloves, powder free	0.38 (finger) 0.36 (palm)
Encore	Smith & Nephew Perry	Chemotherapy gloves, latex	0.60
Tactyl-1	Tactyl Technologies SmartPractice	Nonlatex exam gloves (hypoallergenic)	0.13-0.28
Royal Shield	SmartCare	Powder-free surgical gloves (latex)	0.18-0.24
Perry Style 42	Smith & Nephew Perry	White latex surgical gloves	0.39

From Connor et al., 1995[125]

Engineering controls. OSHA guidelines suggest that class II or III BSCs that meet the current National Sanitation Foundation Standard (Standard no. 49 for class II [laminar flow] biohazard cabinetry, 1990; National Study Commission [NSC] on Cytotoxic Exposure, 1984)[48] should minimize exposure to hazardous drugs during preparation. Studies on use of these cabinets have documented reduced urine mutagenicity in cytotoxic drug-exposed workers or reduced environmental levels after the installation of BSCs.[52,88,100]

There are four types of class II BSCs available.[112] They all have downward airflow and HEPA filters. They are different by the amount of air recirculated within the cabinet, whether this air is vented to the room or the outside, and whether contaminated ducts are under positive or negative pressure:

1. Type A cabinets recirculate approximately 70% of cabinet air through HEPA filters back into the cabinet; the rest is discharged through a HEPA filter into the preparation room. Contaminated ducts are under positive pressure.

2. Type B1 cabinets have higher-velocity air inflow, recirculate 30% of the cabinet air, and exhaust the rest to the outside through HEPA filters. They have negative-pressure contaminated ducts and plenums.

3. Type B2 systems are similar to type B1 except that no air is recirculated.

4. Type B3 cabinets are similar to type A in that they recirculate approximately 70% of cabinet air. However, the other 30% is vented to the outside, and the ducts are under negative pressure.

Class III cabinets are totally enclosed with gas-tight construction. The entire cabinet is under negative pressure, and operations are performed through attached gloves. All air is HEPA filtered.

Based on the recent information regarding the potential for cyclophosphamide and possibly other antineoplastic agents to volatilize off the HEPA filter, it is recommended that the class II B or class III BSCs be used since they are vented to the outside.[123] ASHP recommends that BSCs be serviced and certified by a qualified technician every 6 months or any time the cabinet is moved or repaired (ASHP, 1990; NSC on Cytotoxic Exposure. Recommendations, 1983).[48, 123] Technicians servicing these cabinets or changing the HEPA filters need to be aware of hazardous drug risks through hazard communication training from their employers and should use the same PPE as recommended for large spills.[133-135]

Good handling practices. Correct work practices are necessary for worker protection. Aseptic techniques are very important in drug preparation. When handling vials, extremes of positive and negative pressure in medication vials should be avoided. This situation can occur, for example, when attempting to place 10 mL of fluid (e.g., sterile water) into an air-filled vial of an antineoplastic drug for reconstitution or when attempting to withdraw 10 mL of fluid from a 10-mL vial. Multiuse dispensing pins are recommended to avoid these types of problems.[48]

Decontamination of all work surfaces should be conducted at the end of each work shift at a minimum. Currently the work surfaces of many hospital pharmacies and outpatient cancer treatment clinics (e.g., infusion therapy) handling antineoplastic agents

use protective absorbent pads. All solid surfaces (e.g., carts, trays or baskets, and bench tops) are usually cleaned at the end of each day using towelettes containing either alcohol or phenol. These agents appear to be effective in removal of the antineoplastics that may be present (based on their solubility). Other solutions effective for removal include sodium hydroxide (also used by some researchers as a sorbent for wipe sampling surfaces) and bleach (sodium hypochlorite). The bleach appears to be one of the better solutions for decontamination of most antineoplastic drugs but not all (it is an effective decontaminant for the five antineoplastics in this study). Cleaning solution containing bleach and methanol is used at the end of each day to clean and decontaminate all surfaces potentially contaminated by antineoplastics.[136]

Information on existing exposure monitoring methods

A variety of methods exist for monitoring exposures to antineoplastic drugs.[136-140] These methods vary from biological monitoring, both *in vitro* and *in vivo*, to physical monitoring of air or surfaces in an area where agents of concern may be handled. Biological monitoring may be used to determine the actual concentration of a specific agent in a person's body, such as cyclophosphamide content in certain body fluid(s) (e.g., cyclophosphamide in blood or urine) or an effect on a biological mechanism (e.g., SCE, effects on DNA and/or messenger RNA). Biological monitoring is an indication of whether or not exposure has actually occurred.[140-147]

External or environmental monitoring involves a specific type (or types) of sample collection methods (e.g., air sampling, surface sampling) and a specific type of analysis of the sample(s) (e.g., gas chromatography-mass spectroscopy [GC-MS], reverse-

phase high performance liquid chromatography [HPLC]).[148-151] This type of monitoring is used to determine the concentration of an agent present that could present an exposure to individuals working in an area.

Biological monitoring and associated results. Some researchers believe the basis of controlling carcinogens is the ability to identify chemicals that are potentially carcinogenic to humans in the human.[30] Analytical methods are available for the determination of anticancer agents from biological samples. These methods may include HPLC with ultraviolet (UV) detector, GC-MS, fluorometry, chemical ionization-mass spectroscopy (CI-MS), and GC.

Surveillance of chromosomal damage in human somatic cells, in practice meaning analysis for structural chromosome aberrations (CA), SCE, or micronuclei (MN) in peripheral blood lymphocytes cultures, has been used as a biological monitoring method for genotoxic exposure.[30,146]

On the basis of data on the urinary excretion of cyclophosphamide by health care workers, Sessink et al.[147] estimated the uptake of cyclophosphamide and calculated an additional cancer risk from systemic exposure. In a group of eight hospital pharmacy technicians involved in the preparation of antineoplastic drugs, urine samples were collected during two to four periods of 4 days successively. A total of 476 urine samples were analyzed, and cyclophosphamide was detected in one or more urine samples of each worker. The mean daily excretion rate of 0.18 ug (range: 0.01-0.53 ng) of cyclophosphamide was found.[61] The corresponding calculated uptake varied from 3.6 to 18.0 ug of cyclophosphamide per day, resulting in an additional cancer risk of 1.4 to 10.0

cases per million workers per year. In a group of seven nurses involved in the administration of antineoplastic drugs, a mean excretion rate of 0.80 ug of cyclophosphamide per day was found. This almost fivefold higher excretion rate may represent a corresponding higher uptake of cyclophosphamide (16 to 80 ug per day) and thus an additional cancer risk (7 to 50 cases per million workers per year). Because the environmental contamination found in the present study tends to be higher than that reported by Sessink et al.,[16] Canadian and U.S. health care workers would seem to be in danger of slightly higher additional risks for cancer. As the number of cancer patients increases and higher dosages are used (a benefit of biological response modifiers), the overall amount of chemotherapy use will increase, resulting in an even greater potential for exposure.

Existing methods for analysis of environmental samples

One of the primary objectives of this study was to develop an analytical method or enhance an existing one for the simultaneous identification of a number of different antineoplastic drugs.[148-150] The different agents were: cyclophosphamide, ifosfamide, 5-fluorouracil, doxorubicin-HCl, and paclitaxel (taxol).

The first step in this attempt was to evaluate existing methods for these agents, that is, methods that have already been validated and used in field tests. Methods identified as fitting these criteria are indicated in the following paragraphs with cyclophosphamide the agent of primary interest.

Cyclophosphamide analysis. Because of the similarity in chemical structure, a method for cyclophosphamide is also acceptable for ifosfamide. This method can be

used for either air samples or wipe samples and involves GC-MS (gas chromatography-mass spectrometry) analysis.[16] The samples are centrifuged and a buffer (TRIS buffer, pH 4.5) and an internal standard (5 mg ifosfamide/mL distilled water) are added. After mixing, the samples are extracted twice with 5 mL ethyl acetate, and the ethyl acetate layers are combined in conical tubes with screw caps. The extracts were dried under nitrogen and mixed with ethyl acetate until the residue is totally dissolved. After additional manipulation (further derivatisation and cooling, etc.), the samples are stored at –20 °C until analysis. The lower detection limit for cyclophosphamide using this GC-MS method was <0.1 ng/cm^2 on hood surfaces, and 0.1 μg/L in urine (or 0.1 ng/mL).[16]

A similar method involving GC-MS-MS (gas chromatography in tandem with mass spectroscopy-mass spectroscopy) has also been used for cyclophosphamide analysis.[19] The analytical limit for cyclophosphamide (and ifosfamide) using this method was 0.1 ng/mL of extract, which is similar to the GC-MS method described above. These gas chromatographic methods are commonly used for determination of cyclophosphamide in biological materials.[60,67] But, the methods do have some restrictions; they require complicated sample handling and often require derivatisation in order to avoid the decomposition of cyclophosphamide on the column. The application of HPLC has been recommended for determination of cyclophosphamide, which easily may be degraded on GC columns.

The HPLC method allows direct analysis of cyclophosphamide, which is readily water soluble. In the 1980s, the detection limit of this method was generally considered to be 100 ng/injection or 1.0 μg/m^3 air sample volume.[60] The recovery from the filter was good (97%), when compared to earlier studies indicating only 75% recovery for

cyclophosphamide from filters.[14] In stability tests, Pyy et al. also noted that in 8 days the concentration of cyclophosphamide only decreased 15% on the filter and 25% in water extract. Subsequent to the work noted above, because of enhancements in HPLC column technology and computer enhancements, the lower limit of detection for reverse-phase HPLC is currently as low as 0.05 μg/mL of solvent.[152-154]

Existing air monitoring methods for cyclophosphamide and related agents

In early monitoring work by A. deWerk Neal et al.,[15] 3-month air-sampling program for cyclophosphamide, 5-fluorouracil, and doxorubicin was conducted. Two-week air-sampling efforts were made in the medicine-preparation room, examining rooms, and offices for physicians and nurses. Antineoplastics were prepared daily at a counter in the medicine-preparation room, which was a small room with no windows, one door, and one vent connected to a central heating and air-conditioning system.

Air sampling was conducted by using a portable pump operated at 1-4 L/min., connected to an open-faced 47-mm cassette, which housed a 47-mm, 0.5-μm Teflon filter. The filter cassette was mounted at breathing-zone height above the counter where the worker prepared antineoplastics. Preliminary estimates of possible drug loss were 1 part in 10^4 to the work-area air and the relatively low flow rate of the sampler; 40-hour collection periods per filter (8 hours per day, 5 days per week) were initially specified. Subsequently, 80-hour collection periods (two 40-hour filters extracted together) were found to be more appropriate for collecting an adequate mass of drug for analysis. In order to report volumetric flow rates at standard conditions, temperature and barometric pressure were recorded at the beginning and end of each 40-hour collection period. Filters were

handled with forceps to minimize contamination and were stored in a freezer in plastic Petri dishes taped around the side until extraction and analysis.[15]

The filters were extracted with 1mL filtered distilled water, acidified to pH 2.5 with phosphoric acid, and agitated in a 25 °C water bath for 30 minutes at 80 oscillations per minute. From filters spiked with known drug amounts, essentially 100% of the 5-fluorouracil was recovered and approximately 75% of cyclophosphamide was recovered. Separate aliquots (0.1 mL) of the extract were injected directly onto a μBondapak C_{18} (Waters Associates, Milford, MA) reverse-phase column for HPLC determination of each of the drugs. The mobile phase for CP was 25% acetonitrile and 75% 0.12 *M* $NH_4H_2PO_4$ acidified with 0.2% H_3PO_4 with a retention time of 6 minutes. The mobile phase used for 5-fluorouracil was 0.05% H_3PO_4 in water (pH 2.5), and the retention time was 2.7 minutes. The mobile phase for doxorubicin contained 60% (v/v) methanol, 39.2% water, and 0.8% acetic acid, and the retention time was 5.1 minutes.[15]

A selectable-wavelength UV detector set at 254 nm was used for 5-fluorouracil analysis, and the same detector set at 195 nm was used for cyclophosphamide analysis. Doxorubicin was monitored with a variable wavelength fluorescence detector with excitation at 239 nm and emission cut-off of 550 nm. The minimum detection limits for cyclophosphamide, 5-fluorouracil and doxorubicin were 0.06, 1.0, and 30 ng on column, respectively, and the coefficient of variation of the assays was 2-4%. Of interest relative to this study is that only 1 air sample in 14 detected cyclophosphamide, even though the actual amount of cyclophosphamide handled during the monitoring ranged from 7,400 to 29,900 mg during the sample collection period. Results were a bit higher for 5-

fluorouracil with it being detected in 9 of 14 samples, with the actual amount of 5-fluorouracil handled ranging from 18,500 to 74,000 mg per sample period.[15]

In 1993, Bristol-Myers Squibb Company developed a method for monitoring cyclophosphamide in air using Teflon filters.[152] This method involved the use of 0.45-μm, 37-mm Teflon filters with support pads (SKC Inc., catalog # 225-17-04, or equivalent) for dust sample collection. The filter cassettes used to house the Teflon filters were two-piece, 37-mm size with plugs (Nucleopore SN: 300061, Lot No. 1168 and 1650, or equivalent). However, no actual samples were collected. The filters used in the method development were preloaded to yield concentrations of approximately 1.04 and 5.20 μg/filter. The aliquots were air dried on the filters, four at each concentration, and the dried filters were extracted *in situ* with HPLC grade water (Milli-Q or equivalent). These filters were used for testing recovery (desorption efficiency), system reproducibility, and stability.

The analysis involves use of a reverse-phase HPLC for determination of the cyclophosphamide collected on the Teflon filters. This HPLC system is equipped with a pump set at a flow rate of 0.8-mL/minute; an auto sampler for injection of 50 μL per analysis; a ultraviolet-visible spectrophotomer (UV-VIS) detector (or visible based analysis) set at wavelength of 200 nm with sensitivity (analog chart recorder output) set at 0.5 absorbance units per volt (AU/V). It uses a Nova-pak C-18, 60 Å, 4 μm, 3.9 x 150 mm (spherical) Waters analytical column, with column oven temperature of 35 °C The mobile phase is acetonitrile:10 mM potassium phosphate buffer (pH 6.0) at a ratio of 25:75. Typical retention time for this method is 4.6 minutes.[152]

The sample filters when received at the lab are stored in the sampling cassette. For analysis, the plug from the top aperture of the cassette is removed, and 2.0 mL of Milli-Q or equivalent water is accurately delivered into the cassette. The plug is replaced, and the cassette is shaken with a reciprocating shaker for about 15 minutes to assure the cyclophosphamide in the sample is completely dissolved. The prepared sample is assayed using the HPLC system described above. Cyclophosphamide standard is injected initially and at a maximum of every 10 sample sets for accuracy in quantification of cyclophosphamide determined to be present. If necessary, samples can be diluted with the mobile phase reagent to accommodate the upper concentration limit of the standard(s).[152]

The cyclophosphamide standard (stock) solution is prepared by accurately weighing 10.0 ± 0.05 mg cyclophosphamide reference standard in duplicate and dissolving each weighing in 10 mL volumetric flasks, thus, providing two flasks of standard solution (A and B). Bristol-Myers Squibb indicated that this stock standard is stable for 1 week when stored at 4 °C.[152]

A 1.0-mL aliquot of flask is delivered to a 10 mL volumetric flask and diluted with methanol:water at a ratio of 50:50 and mixed well. This is called the primary stock solution A and is further blended with water to prepare working standard solutions with cyclophosphamide concentrations of 0.05 μg/mL, 0.1 μg/mL, 0.25 μg/mL, 0.5 μg/mL, 2.5 μg/mL, 5.0 μg/mL, and 10.0 μg/mL.[152]

The concentration of cyclophosphamide present in a sample is calculated using a standard curve for cyclophosphamide standards. These curves are prepared by plotting average peak height responses observed for the working standards that bracket the re-

sponses observed for the sample against the theoretical concentration of the respective standard(s). The concentration of cyclophosphamide in a sample is determined from the standard curve, and the result is multiplied by the appropriate factors to correct for sample dilutions and for purity of the cyclophosphamide standard.[152]

Cyclophosphamide is observed as a symmetrical peak at a retention time of 4.6 minutes, with no interfering peaks appearing in the chromatogram. However, when blank and spiked Teflon filters were prepared for analysis according to the procedures indicated above, three unknown peaks appeared from the analysis of the blank extract at retention times of 2.85, 3.25, and 3.95 minutes. Fortunately, these did not interfere with the cyclophosphamide peak, but do need to be considered when this method is being used to analyze for additional antineoplastic agents.

A linear regression curve was constructed by plotting average peak height responses observed from using injections of cyclophosphamide in six standard solutions at 0.052 μg/mL, 0.104 μg/mL, 0.260 μg/mL, 0.520 μg/mL, 2.596 μg/mL, and 5.191 μg/mL. Bristol-Myers Squibb reported that the standard curve was linear for the concentrations in the range of 0.052 to 5.2 μg/mL, with a correlation coefficient (R^2) of 0.99999, Y-intercept of 21.2, and slope of 2742.[152]

Instrument precision was evaluated using six injections for each of the same standard solutions as indicated above. The coefficient of variation (C.V.) for the six consecutive injections of a 0.52 μg/mL solution of cyclophosphamide, which is a concentration equivalent to the proposed exposure limit of 1.0 μg/sample, gave and acceptable precision of 0.3%. At a concentration of 0.052 μg/mL, an acceptable C.V. of 3.1% was obtained for six consecutive injections.[152]

Cyclophosphamide recoveries were acceptable at 99.0% of theory with a C.V. of 0.9% for the filters having 1.04 μg each and 99.4% of theory with a C.V. of 0.9% for the filters having 5.20 μg each. The average recovery from filters for all concentrations tested was 99.2% of theory with a C.V. of 0.9%. Testing was also conducted to determine if there was any interference from methanol, which is used in the stock standard (but only water added to form the working standards). The test involved comparison of spiked filter extractions, one using only water and another using water with a small amount of methanol added. The average peak height response was 5590 units for spiked filter extracted in pure water versus a peak height response of 5580 units for spiked filter extracted in water containing methanol. This indicates the presence of trace amounts of methanol and has no significant effect on the UV response observed for cyclophosphamide.[152]

The minimum detection limit (MDL) of cyclophosphamide for this assay is 0.014 μg/mL, which is equivalent to 0.03 μg/sample. MDL is estimated at twice the base line noise.[152]

Stability tests. Stability tests were conducted on this analytical method for cyclophosphamide using standard solutions of 0.52, 2.60, and 5.20 μg/mL, each prepared in water (Bristol-Myers Squibb). These solutions were maintained at room (ambient) temperatures and analyzed at days 0, 1, and 3. Each day's analytical results were compared to freshly prepared standard. The cyclophosphamide was stable for a period of 3 days for all concentrations tested, with recoveries ranging from 96% to 100%, when solutions were maintained at room temperature.[152]

Stability tests were also conducted by Bristol-Myers Squibb on 12 Teflon filters spiked with 5.19 μg of cyclophosphamide per filter and air-dried. Two filters were stored at −20 °C for 14 days, and 10 filters were stored at room temperatures for 0, 7, 14, and 30 days. Cyclophosphamide was recovered at an average of 99.4% of theory for spikes prepared and assayed on the same day. Storage of spikes at room temperature for 7, 14, and 30 days resulted in a gradual loss of cyclophosphamide potency. Calculated as a percent of theory, the potency loss ranged from approximately 8% after 7 days to 32% after 30 days. Spiked filters stored at −20 °C for 14 days were recovered at 105% of theory. This indicates samples should be stored at −20 °C and assayed as soon as possible after they are collected to avoid error due to loss of potency.[152, 155-157]

General information on cyclophosphamide air monitoring. As indicated above, cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. It is usually packaged as a white crystalline powder and is soluble in water, saline, or ethanol. Its molecular weight is 279.1, and it has a melting point of between 41 °C and 45 °C. Prior air monitoring methods have utilized 0.45 μm Teflon filters for sample collection. Stability tests on samples collected by the Teflon filter method are indicated to be very good for periods of up to 30 days when spiked with 5.19 μg/filter (Bristol-Myers Squibb). However, these are spiked filters and are maintained in tightly closed filter cassettes. Thus, vaporization is not able to be considered, either that which might occur during a routine monitoring period or that which might occur if the filter was left in an open container.[152, 155-157]

Because of its frequent use and its carcinogenic properties, the interest in monitoring for cyclophosphamide is very high. Therefore, primary sample collection development will be on cyclophosphamide. Differences in the methods have been in size and type of sampling media and in flow rate and total sample volume. To date, all sample systems for cyclophosphamide have used the filter method; however, the type of filter has varied from glass fiber to Teflon. Most current studies use a 0.5 μm (or 0.45 μm) pore Teflon filter. Size of filters and respective cassettes vary from 25 mm to 47 mm, with the 37 mm appearing to be most often used. Flow rates currently vary from 1.0 to 4.0 L/minute, and sample period may vary from a minimum of 40 hours (usually 5 days at 8 hours per day with the cassette wrapped in cellophane when not sampling). Some studies indicated 40 hour samples to be inappropriate for collecting an adequate mass of drug for analysis. These types of sample method can be used for either individual or area/source monitoring purposes.

A similar method involves use of high volume air sampling and has been used to evaluate airborne concentrations of cyclophosphamide in oncology pharmacy and clinic areas.[18] In this method, air samples are collected at fixed locations where cyclophosphamide is prepared or administered. The sample sites include inside and immediately outside the BSC, and near waste disposal area(s). Air samples are collected on 25-mm Type A/E glass fiber filters (Gelman Sciences). Air is drawn through the filters at 13-15 L/min using calibrated high volume pumps. Sampling is conducted 24 hours/day for 19 consecutive days at each sample point. After collection, each sample filter is placed in individual glass storage vials and stored at –40 °C until subsequent analysis.

Ifosfamide air monitoring and analysis. Ifosfamide is a structural isomer of cyclophosphamide with one of the chloroethyl side chains residing on the ring. Bristol-Myers Squibb Company developed a method for monitoring ifosfamide in air using Teflon filters, which is very similar to their method for monitoring cyclophosphamide in air. This method involved the use of 0.45 μm, 37-mm Teflon filters with cellulose support pads (SKC Inc., catalog # 225-17-04 or equivalent) for dust sample collection. The filter cassettes used to house the Teflon filters were two-piece, 37-mm size with plugs (Nucleopore SN: 300061, or equivalent). However, no actual samples were collected. The filters used in the method development were preloaded to yield 6.00 μg/mL, 60.0 μg/mL, and 600.0 μg/mL for testing recovery (desorption efficiency), system reproducibility, and stability, respectively.[152]

The analysis involves use of a reverse-phase HPLC for determination of the ifosfamide collected on the Teflon filters. This HPLC system is equipped with a pump set at a flow rate of 1.5-mL/minute, an auto sampler for injection of 50 μL per analysis, a UV-VIS detector set at wavelength of 200 nm. It uses a Whatman Partisil 5 ODS-3 Cartridge System 125 mm x 4.6 mm ID analytical column, and the mobile phase is MeOH:H_2O at a ratio of 45:55. Typical retention time for this method is 2.4 minutes.[152]

The sample filters when received at the lab are stored in the sampling cassette. For analysis, the plug from the top aperture of the cassette is removed, and 5.0 mL of mobile phase reagent is accurately delivered into the cassette. The plug is replaced, and the cassette is shaken with a reciprocating shaker for about 15 minutes to assure the ifosfamide in the sample is completely dissolved. The prepared sample is assayed using the HPLC system described above. Ifosfamide standard is injected initially and at a maxi-

mum of every 10 sample sets for accuracy in quantification of ifosfamide determined to be present. If necessary, samples can be diluted with the mobile phase reagent to accommodate the upper concentration limit of the standard(s). [152]

The ifosfamide standard (stock) solution is prepared by accurately weighing 60.0 ± 0.2 mg ifosfamide reference standard in duplicate and dissolving each weighing in 10 mL of mobile phase to provide two stock solutions (A and B). Stock solution A is used to prepare the standard curve and spike standards and spike controls. Stock solution B is diluted to a 60.0-μg/mL solution and is analyzed to confirm the accuracy of the standards prepared from stock solution A. [152]

The concentration of ifosfamide present in a sample is calculated using a standard curve for ifosfamide standards. These curves are prepared by plotting average peak height responses observed for the standards, which bracket the responses observed for the sample against the theoretical concentration of the respective standard(s). The concentration of ifosfamide in a sample is determined from the standard curve, and the result is multiplied by the appropriate factors to correct for sample dilutions and for purity of the ifosfamide standard (plans are to use research grade or near 100.0 % ifosfamide). [152]

This method has been reported by Bristol-Myers Squibb, based on seven consecutive injections of 6.0 μg/mL, 60.0 μg/mL, and 600.0 μg/mL solutions of ifosfamide, as giving an acceptable precision with a C.V. of 1.2%, 0.21%, and 0.5% respectively. Ifosfamide recoveries were acceptable with an average recovery of 100.6% of theory and C.V. of 1.1%. The MDL of ifosfamide was established to be 2.3 μg per filter. [152]

Stability tests of ifosfamide in mobile phase at ambient temperatures were conducted at zero hours and 24-hour intervals to 72 hours. Ifosfamide dissolved in mobile

phase was stable at ambient temperature for all concentrations tested. Earlier stability tests were found acceptable on the Teflon filters. Additional stability testing was conducted on glass fiber filters, storing the filters at 4 °C and assaying the samples after 3, 5 and 9 days. Results indicated ifosfamide to be stable for at least 9 days on glass fiber filters when stored at the temperature indicated.[152]

For this study, it is planned that ifosfamide will be analyzed by the same methodology as that used for cyclophosphamide. The challenge may be in differentiating the ifosfamide from the cyclophosphamide, but there may be sufficient difference due to the H_2O attached to the cyclophosphamide to have separation of the two agents in HPLC analysis. Another example is an HPLC method previously used to analyze ifosfamide in aqueous buffers.[65]

5-Fluorouracil analysis. One analytical method used for the 5-fluorouracil involves use of a reverse-phase HPLC system. Twenty-microliter aliquots of the centrifuged extracts are injected onto a 150 x 4.6 mm Nucleosil RP 18 column with 5-μm particles. The column temperature is 40 °C. A sodium acetate buffer (0.05 M; pH 4.0) is used. Solvent flow rate is 1.0 ml/minute and the apparatus is equipped with a UV spectrophotometer. 5-Fluorouracil is analyzed at 260 nm with a retention time of 3.5 minutes. The selectivity of the method has been verified by the analysis of blank and spiked samples and by taking full UV spectra. Quantification is carried out by reference to the calibration curves constructed from the analysis of freshly prepared reference samples containing 5-fluorouracil dissolved in distilled water. Limits of detection for this method are indicated to be approximately 7 μg/L sample.[16]

Another method for determination of fluorouracil content of a filter extract, which used water as the desorbent, was conducted by Hoffman et al. (1980)[158] using the HPLC methodology. A Waters Radial-pak μBondapak C18 reverse-phase column, an HPLC pump, a UV detector, a gradient controller, and a data module (all from Waters Associates, Milford, MA) were used. The mobile phase was an aqueous system adjusted to a pH 2.5 with phosphoric acid. The system was operated isocratically with a flow rate of 2.0 mL/minute. The detector was set at 254 nm. The attenuation for the detector was set at 0.005. The detection limit was determined to be 1 ng.

It is interesting to note that no airborne fluorouracil was detected in the preparation area adjacent to the BSC. The negative control was also found to be without detectable levels of fluorouracil. In the first sampling period (56 hours), five persons prepared 25.4 g of fluorouracil, and 6.28 cu m of air was filtered. In the second sampling (95 hours), four persons prepared 25 g of fluorouracil and 11.51 cu m of air was filtered.

Another reverse-phase HPLC method was used by Stiles et al.[159] to evaluate stability of fluorouracil. HPLC equipment for this method was similar to that described in the method above. That is, it uses a HPLC system consisting of a C18 column, a pump, a UV detector, an integrator, an auto sampler, and an injector. However, conditions were different. In this method the mobile phase consisted of 0.005 M dibasic potassium phosphate, pH 7.8, the UV detector setting was 214 nm, flow rate was 0.5 mL minute with a retention time of 6.6 minutes, and the concentration range for a standard curve was 100 to 1000 μg/mL.

Doxorubicin analysis. Normal analysis for doxorubicin is by HPLC method. As indicated above, an early HPLC method was one used by A. deWerk Neal et al.[14] It involved a 3-month air-sampling program for cyclophosphamide, 5-fluorouracil, and doxorubicin HCl. Two-week (80-hour) air-sampling efforts were made in the medicine-preparation room and other areas associated with cancer therapy centers. The filter extracts from these samples were injected directly onto a μBondapak C_{18} (Waters Associates, Milford, MA) reverse-phase column for HPLC determination of each of the drugs. The mobile phase for doxorubicin contained 60% (v/v) methanol, 39.2% water, and 0.8% acetic acid, and the retention time was 5.1 minutes. Doxorubicin was monitored with a variable wavelength fluorescence detector with excitation at 239-nm and emission cut-off of 550 nm. The minimum detection limits for doxorubicin was 30 ng on-column, and the coefficient of variation of the assays was 2-4%.

A more recent study of doxorubicin was conducted by Stewart et al.[160] The HPLC system that was used consisted of a pump, an auto sampler programmed to deliver a volume of 50 μL, and an appropriate column (e.g., Waters μBondapak phenyl column, 30 cm, 3.9 mm inner diameter, 10 μm particle size). The UV variable-wavelength detector was set at 216 nm for detection of doxorubicin.[160] An electronic integrator was used to measure peak areas. The mobile phases were prepared from HPLC-grade solvents and cartridge purified water. The initial pH of the doxorubicin in 5% dextrose injection was 3.1-3.8, while the pH of doxorubicin in 0.99% sodium chloride injection was 4.4- 4.5.

The relative standard deviation of each HPLC method for replicate injections of the same sample or standard solution of each medication was less than 4%. As for stability, a drug was considered stable if it retained greater than 90% of its time-zero concen-

tration at the end of 24 hours at 30 °C or after 7 days when stored at 4 °C. Doxorubicin retained a mean of >90% of the initial concentration in both cases. However, the stability of doxorubicin will vary based on the type of container it is stored in and any other agents it may come into contact with.[160]

Paclitaxel (Taxol) analysis. One analytical method for Taxol uses a reversed-phase HPLC system and a pentafluorophenyl reversed-phase column (4.0 mm x 25 mm, 5 µm, ES Industries, NJ). The mobile phase consisted of methanol:acetonitrile:50 mM ammonium acetate (20:32:48) and is adjusted to pH 4.4 with acetic acid.[73] The UV detector setting was 230 nm.

Another reversed-phase HPLC method for paclitaxel uses a system with an auto sampler and a diode array UV detector. A Phenomenex Curosil pentafluorophenyl (PFP) column, 25 cm x 4.6 mm inside diameter, 5 µm particle size, from Phenomenex (Torrance, CA) is used in the system. The detector wavelength used is 230 nm, the column is operated under ambient temperatures, and injection volume is 15 µL.[161] This method uses a linear solvent gradient from 40/60 ACN (Acetonitrile)/water (v/v) at 0.444%/minute until the paclitaxel peak is eluted (~24 minutes). That is followed by rapid return to the initial composition and equilibration prior to the next injection. The separation of up to 15 taxanes, including paclitaxel using this method, is less than 30 minutes.[162]

An alternate method uses a Whatman TAC-1 column with an initial 12 minutes isocratic 38/62 ACN/water (v/v), followed by a linear gradient at 4.0%/minute until the paclitaxel peak eluted (~17 minutes), and then a return to the initial composition and equilibrium. The flow rate on this method is 1.5 mL/minute, and in addition this column

is less retentive. The effect of this method on peak width is that the peaks broaden up to ~12 minutes, as usual for isocratic run; once the gradient starts, the peak widths shrink, and apparent plate numbers increase dramatically.[161] This method is able to separate 15 taxanes, including paclitaxel, in less than 20 minutes.[161]

Both of the above methods, using an aqueous acetonitrile gradient, found paclitaxel well resolved from the other taxanes.[161, 162] It was indicated by Shao,[161] the developer of the method, to be sensitive, selective, accurate, precise, rugged, and stability indicating. The methods were fully validated for the analysis of paclitaxel in the bulk drug form.

Bristol-Myers Squibb, using Teflon filters for sample collection, has also developed a reverse-phase HPLC analytical method for determination of Taxol in dust.[162] This method involves the use of 0.45-μm, 37-mm Teflon filters with support pads (SKC Inc., catalog # 225-17-04, or equivalent) for dust sample collection. The filter cassettes used to house the Teflon filters were two-piece, 37-mm size with plugs (Nucleopore SN: 300061, Lot Nos. 1168 and 1650, or equivalent). However, no actual samples were collected. The filters used in the method development were preloaded to yield Taxol levels of 0.823 and 3.29 μg/filter. The aliquots were air dried on the filters, four at each concentration, and the dried filters were extracted *in situ* with acidified methanol. This diluent is prepared by adding 200 μL of glacial acetic acid to a 1-L volumetric flask and filling the flask to the mark with methanol and then mixing well. The above spiked filters are used for testing recovery (desorption efficiency), system reproducibility, and stability.[163]

The analysis involves use of a reverse-phase HPLC for determination of the Taxol collected on the Teflon filters. This HPLC system is equipped with a pump set at a flow rate of 1.5-mL/minute, an auto sampler for injection of 20 µL per analysis, a UV-VIS detector set at wavelength of 227 nm with sensitivity set at 1 AU/V. It uses a chromega-bond pentaflurophenyl (PFP), 5 µm, 15 cm, 4.0 mm inside diameter, 60 Å column. The mobile phase is acetonitrile:water at a ratio of 50:50. Typical retention time for this method is 3.3 minutes.[163]

The sample filters when received at the lab are stored in the sampling cassette. For analysis, the plug from the top aperture of the cassette is removed, and 2.5 mL of acidified methanol is accurately delivered into the cassette. The plug is replaced, and the cassette is shaken with a reciprocating shaker for about 30 minutes to assure the Taxol in the sample is completely dissolved. If insoluble particulates are observed, the samples need to be centrifuged prior to analysis.[163]

The prepared sample is assayed using the HPLC system described above. Taxol standard is injected initially and at a maximum of every 10 sample sets for accuracy in quantification of Taxol determined to be present. If necessary, samples can be diluted with the mobile phase reagent to accommodate the upper concentration limit of the standard(s).[163]

The Taxol standard (stock) solution is prepared by accurately weighing 10.0 ± 0.05 mg Taxol (Bristol-Myers Squibb -181339-01) reference standard in duplicate and dissolving each weighing in 25 mL volumetric flasks and diluting to volume with acidified methanol, thus, providing two flasks of standard solution (A and B). Bristol-Myers Squibb indicated that this stock standard is stable for 1 week when stored at 4 °C.[163]

A 1.0 mL aliquot of flask is delivered to a 5 mL volumetric flask and diluted with acidified methanol and mixed well to prepare a primary stock solution A. This solution is further blended with acidified methanol to prepare working standard solutions with Taxol concentrations of 0.033 μg/mL, 0.165 μg/mL, 0.329 μg/mL, 1.646 μg/mL and 3.292 μg/mL.[163]

The concentration of Taxol present in a sample is calculated using a standard curve for Taxol standards. These curves are prepared by plotting average peak height responses observed for the working standards that bracket the responses observed for the sample against the theoretical concentration of the respective standard(s). The concentration of Taxol in a sample is determined from the standard curve, and the result is multiplied by the appropriate factors to correct for sample dilutions and for purity of the Taxol standard (plans are to use research grade or near 100% Taxol).[163]

Taxol is observed as a symmetrical peak at a retention time of 3.1 minutes, with no interfering peaks appearing in the chromatogram. However, when blank and spiked Teflon filters were prepared for analysis according to the procedures indicated above, two unknown peaks from the blank extract at retention times of 4.2 and 4.7 minutes. Fortunately, these do not interfere with the Taxol peak but need to be considered when this method is being used to analyze for additional antineoplastic agents.[163]

A linear regression curve was constructed by plotting average peak height responses observed from using injections of cyclophosphamide in six standard solutions at 0.033 μg/mL, 0.165 μg/mL, 0.329 μg/mL, 1.646 μg/mL, and 3.292 μg/mL. Bristol-Myers Squibb reported that the standard curve was linear for the concentrations in the

range of 0.052 to 5.2 μg/mL, with a correlation coefficient (R^2) of 0.99999, Y-intercept of 21.2, and slope of 2742.[163]

Instrument precision was evaluated using six injections for each of the same standard solutions as indicated above. The C.V. for the six consecutive injections of the above concentrations of Taxol were 3.4, 0.8, 0.3, 0.1, and 0.2, respectively. Taxol recoveries were acceptable at 97.8.0% for the filters with 0.82 μg each and 103.2% of theory for the filters having 3.29 μg each. The average recovery from filters for all concentrations tested was 100.5% of theory with a C.V. of 3.8%. The MDL of Taxol for this assay was 0.008 μg/mL, which is equivalent to 0.02 μg/sample. MDL is estimated at twice the base line noise.[163]

Stability tests of Taxol standard solutions of 0.329 and 3.292 μg/mL, each prepared in acidified methanol, were maintained at room (ambient) temperature and at 10 °C. They were analyzed at days 0, 1, 2, and 7. Each day's analytical results were compared to freshly prepared standard. The Taxol was found to be stable for a period of 7 days for all concentrations tested, when solutions were maintained at 10 °C and/or at room temperature. Stability tests were conducted on 10 Teflon filters spiked with 3.3 μg of Taxol per filter, air dried, and stored at room temperature to provide four filters for day 0 and duplicate filters for day 7, 14, and 30, stability samples. Taxol was stable at room temperature for the entire period with recoveries ranging from 103% to 106%.[163]

Existing methods for surface sample collection

A variety of methods have been used for collection of surface samples. They all use wipe methods but vary in the total surface area to wipe, the size and type of wipe

sampling material, the type of solution to use for wetting the wipe sampling material, and the volume of solution for desorption of the sample material. One method developed by Britol-Myers Squibb uses Whatman #1 (qualitative) filter paper circles, 5.5 cm, and only from newly opened boxes. To begin, the individual conducting the wipe testing dons gloves, rinses the gloves with water, and then thoroughly dries the gloves before proceeding. The filter paper is wetted with Milli-Q (or equivalent) water. The circle is folded twice to form a 1/4 circle, and then it is dampened with Milli-Q water, squeezing out any excess water. A 10 x 10-cm surface area is thoroughly swabbed. For quality control (QC) blanks, four filter paper circles are folded and wetted exactly like above, but no surfaces are wiped. Each individual folded filter paper is placed in a clean, dry 2 oz wide-mouth amber jar, and the jars are tightly capped. The samples are now ready for analysis.[154]

Another method developed by Britol-Myers Squibb for surface monitoring of TAXOL on stainless steel and floor tile surfaces involves use of a cotton ball as the wipe material. The cotton ball is wetted with methanol and a 100 cm^2 area is swabbed using vertical strokes. The cotton ball is then rolled over and the same area is wiped using horizontal strokes. The cotton ball is then rinsed with a specific quantity of methanol, excess methanol squeezed into a test tube and the wipe is repeated with the same cotton ball. After this wipe, the cotton ball is placed into the test tube containing the methanol rinse, and the methanol desorbate is analyzed for Taxol.[164]

A method used by Sessink et al.[16] uses 20 x 21 cm tissues (Kleenex professional wipes, Kimberly Clark Corp., Koblenz, Federal Republic of Germany) for wipe sampling. The tissue is wetted with a sodium hydroxide solution (0.03 M), and the spots and

objects are swept clean. For the packings, boxes, chamber pots, and urinals, two tissues and 5 mL of the sodium hydroxide solution are used. The floors, working trays of hoods, the tables, and the sink units are cleaned with four tissues and 10 mL of sodium hydroxide solution. For wipe samples taken from the floor, solution was pipetted on the floor rather than wetting the tissues. The detection limits for the wipe samples are: (1) 0.02 ng/cm^2 for CP and 0.1 ng/cm^2 for 5-fluorouracil on the floor and tables, (2) 0.01 ng/cm^2 for CP and 0.04 ng/cm^2 for 5-fluorouracil on working trays of the hoods, and (3) 0.06 ng/cm^2 for CP and 0.3 ng/cm^2 for 5-fluorouracil on boxes and drug vials or ampules before and packings after preparation of drugs. No indication is given as to the actual surface area wiped when using this method.

A method used by Connor et al.[19] measures an area of 4900 cm^2, spreads a solution of 0.03 M sodium hydroxide over the area with a pipette, typically 20 mL, and uses one or two Scott 130 roll towels (Kimberly-Clark Corp.) to wipe the measured surface. The towel (or towels) is stored in a coded, 125 mL plastic screw-top container (Nalge Nunc). Uneven surfaces are sampled by applying 5 mL of the sodium hydroxide solution to the tissue and wiping the surface. For QC purposes, duplicate blanks are prepared for each sampling by adding 20 mL of sodium hydroxide solution to two tissues and placing them in a plastic container for analysis. When analysis can not be accomplished the same day, samples are stored at –40 °C and shipped on dry ice to the lab for analysis. This method for surface sample collection was used for simultaneously determining the presence of three antineoplastic agents: cyclophosphamide, fluorouracil, and ifosfamide. The lower limit of detection for these agents is 0.01, 0.7, and 0.01 ng/cm^2 respectively.

Goals and objectives of this research

As already indicated, there are a number of activities carried out by both oncology pharmacists and nurses that can lead to their being exposed to antineoplastic (potentially cytotoxic) agents. These activities, because of possible contamination to air or surfaces in the work area, may also result in exposure to individuals not directly involved in the handling of these potentially hazardous agents. A number of studies evaluating exposures to health care workers have been previously conducted. These studies used various methods for detection and analysis of exposures to the various antineoplastic agents to which the health care workers may be exposed. However, some exposure monitoring methods, especially relative to exposure to airborne contaminants, may be inaccurate.

Essentially all airborne contaminant monitoring for antineoplastics to date has assumed particle contamination and involved use of a Teflon or glass fiber filter. New information out of Germany[14] indicated some of the antineoplastic agents may volatilize or sublimate off a filter, whether it be a HEPA filter for BSC use or a filter for sample collection. Thus, it was determined that additional work on monitoring and analysis methodology was warranted.

Study goals. There were two major goals associated with this project. The first is the enhancement of an existing HPLC method of analysis for cyclophosphamide to improve sensitivity and includes acceptable detection and quantification of four other antineoplastic agents: ifosfamide, 5-fluorouracil, doxorubicin, and paclitaxel. The second objective was to develop acceptable methods for collection of samples, specifically in air and on surfaces.

Study objectives. Several objectives were identified to reach these goals. These included the activities listed below by goals.

The first goal (goal 1) was development of an acceptable analytical method for detection of all five agents of interest from the same sample. The primary objectives associated with meeting this goal were: (1) identify operating conditions that would be most likely to produce acceptable results for the simultaneous analysis of five agents, (2) conduct recovery and stability tests for each of the five agents to determine acceptability of the new or enhanced analytical method, and (3) evaluate the analytical method for potential interferences from other agents used in oncology pharmacy or cancer treatment facilities.

The second goal (goal 2) involved development of methods for sample collection. This included development of methods for surface sample collection, and for air sample collection. Objectives associated with meeting goal 2 for wipe sample collection included: (1) identify an acceptable material for use in conducting surface wipe sampling, (2) identify or develop an acceptable reagent for optimum recovery of all five agents in conducting wipe sampling, (3) identify or develop the optimum reagent for desorption of wipe samples from the wipe material used, (4) evaluate the wipe method on three different types of surface materials common to oncology pharmacy or cancer treatment facilities, and (5) evaluate the surface sampling method in oncology pharmacy or cancer treatment facilities to determine potential for interferences.

The objectives associated with goal 2 for air sample collection included: (1) evaluating effectiveness of existing methods used for collection of air contaminant samples; (2) identifying and evaluating alternate air monitoring methods, with an emphasis

on use of solid sorbents for effective collection and acceptable removal (desorption) of all five agents; and (3) evaluation of the air sampling method in oncology pharmacy or cancer treatment facilities to determine potential for interferences,

A couple of objectives were also applied after the analytical and monitoring methods were deemed acceptable. These were: (1) conducting of air and surface monitoring in an oncology pharmacy area and at an infusion therapy clinic to determine potential for occupational exposures in those types of areas, and (2) publish information obtained from this research so that occupational and environmental health professionals may use them to evaluate exposure potentials in areas where antineoplastic agents are or may be used.

Summary of research

Analytical method. For research on the development or enhancement of an analytical method, one of the most important first steps was to determine the ability to detect the agents of interest. And, if able to detect, would the concentrations they could be detected at be low enough to be meaningful? This involved the evaluation of mobile phase solutions that would elute the agents injected and whether to operate in the isocratic or gradient phase or both and the determination of the UV wavelength able to detect all five agents.

As indicated elsewhere in this dissertation, the optimum mobile phase was determined to be 22.75% ACN:77.25 Milli-Q water buffered to pH 6.0. Analyses for four agents (fluorouracil, ifosfamide, cyclophosphamide, and doxorubicin) were accomplished

using the isocratic phase for 20 minutes, but the fifth agent, paclitaxel, required use of an isocratic phase going to 70% ACN in Milli-Q water for an additional 40 minute period.

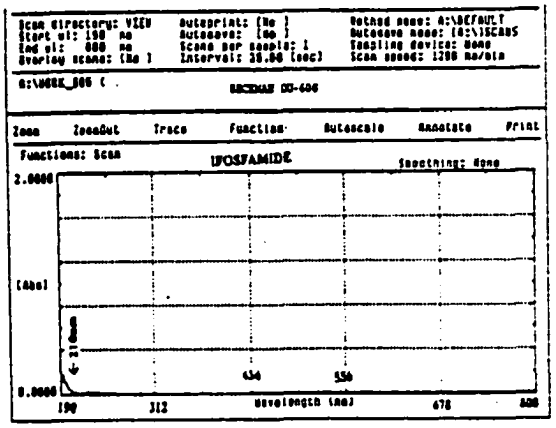
Relative to determination of an acceptable UV wavelength setting for detection, a UV spectrum analysis was conducted using a Beckman DU-600 spectrophotometer. This spectrum analysis was conducted on stock standards of each agent (i.e., in methanol and buffered water). Results of these analyses can be seen in Figure 2.

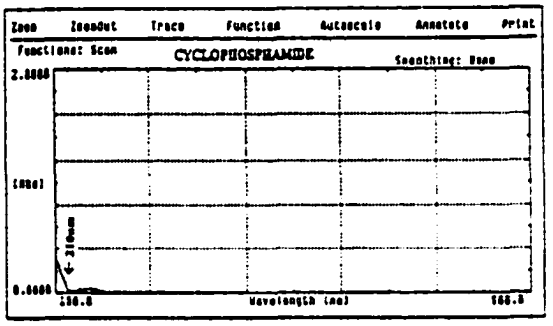
Because of the limited absorption of UV radiation by cyclophosphamide and ifosfamide above 210 nm, 195 nm was used for the optimum wavelength, where all five agents of interest indicated strong absorption. Sensitivity or absorbency under full scale (AUFS) was tested at 0.040 and 0.010 AUFS, with the 0.010 found most acceptable and used for analysis.

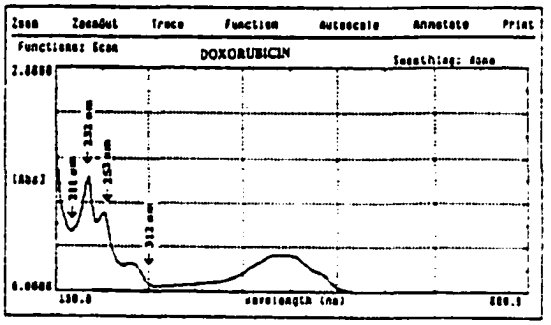
A thorough review of the research and results associated with development of the analytical method can be seen in a the paper titled "Development of an HPLC Method or Simultaneous Analysis of Five Antineoplastic Agents."

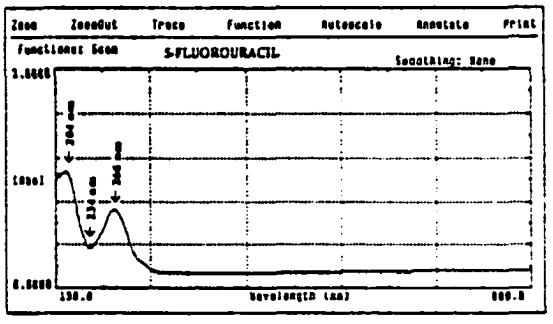
Surface monitoring method. The first priority was to identify different surfaces that may be wiped. Surfaces chosen were stainless steel, vinyl floor tile, and resin counter surfaces. The next step was to choose at least two materials that may be acceptable for the purpose of use as surface wipes. After review of several materials, the two chosen for evaluation were the Whatman #42 ashless circle filters and the Kimwipe EX-L precision wipe sheets. The third step was to develop a reagent for use in wiping surfaces. After a number of trials, a blend of 10% acetonitrile, 25% methanol, and 65%

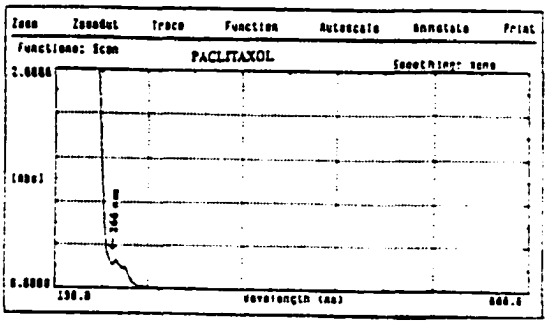
Figure 2. UV spectrum analysis to determine optimum wavelength to detect agents. Results were obtained from a Beckman DU-600 spectrophotometer analysis of agents.











Milli-Q water was determined to be the optimum blend (this appears to be the first time such a blend has ever been used, but it is necessary because of the different chemical characteristics of the agents involved). This blend also was the optimum reagent for desorption of the agents from the wipe materials. The last step was to determine if any interference may be present in an actual cancer treatment or oncology pharmacy that may affect the ability of the collection or analytical method to be less effective. Such testing was conducted on a variety of surfaces in an oncology pharmacy area and at an infusion therapy clinic, with no interferences identified. A thorough review of the research and results associated with development of the analytical method can be seen in the paper titled "Development and Review of a Surface Monitoring Method for Select Antineoplastics."

Air monitoring method. It should be noted that, for an air monitoring method to be considered acceptable, the combined collection and analysis is required to provide results that are within ± 25% of the true concentration 95% of the time and based on worst-case precision and bias estimates of the collected data.

The first priority was to determine effectiveness of the existing monitoring method using filters. It was quickly demonstrated that observations by Schmidt were correct in that the agents, except for paclitaxel, appear to deteriorate (e.g., sublimate) off the filters. The second step was to identify alternate air methods, and the solid sorbent was decided on. After several tests, the Anasorb 708, which is a methyl acrylate polymer sorbent, was chosen. The third step was to develop a reagent for optimum desorption of the agents from the solid sorbent. The blend used for desorption of wipe samples (10%

ACN, 25% methanol, and 65% Milli-Q water) was determined to be the optimum blend for desorption of the Anasorb tubes. The last step was to determine if any interference may be present in an actual cancer treatment or oncology pharmacy that may affect the ability of the collection or analytical method to be less effective. Such air monitoring method testing was conducted in an oncology pharmacy area and at a clinic infusion therapy area, with no interferences identified. A thorough review of the research and results associated with development of the analytical method can be seen in the paper titled "A New Monitoring Method Using Solid Sorbent Media for Evaluation of Airborne Cyclophosphamide and Other Antineoplastic Agents."

Manuscripts submitted for publication

Three manuscripts have been submitted for publication, one on the analytical method developed, one on the surface monitoring method developed, and one on the air monitoring method developed. The manuscript on surface monitoring titled "Development and Review of a Surface Monitoring Method for Select Antineoplastics" has been accepted for publication by the American Journal of Health-System Pharmacists (AJHP Ref Code 7519). The analytical method and surface and air monitoring methods were developed based on both the information indicated above and numerous and various trials conducted in the laboratory. Manuscripts on these two subjects are being submitted to the ACGIH Publication *Applied Occupational and Environmental Hygiene*.

DEVELOPMENT OF AN HPLC METHOD FOR SIMULTANEOUS ANALYSIS OF FIVE ANTINEOPLASTIC AGENTS

by

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Submitted to *Applied Occupational and Environmental Hygiene*

Format adapted for dissertation

ABSTRACT

A variety of acceptable analytical methods are currently available for detection and quantification of antineoplastic agents, but most methods are specific for an individual agent or only applicable for small groups of chemically similar agents. This study describes a reverse-phase high performance liquid chromatograpy (HPLC) method, which is able to identify and quantify a variety of different antineoplastics from a single sample.

It was determined that the ability to simultaneously evaluate five agents was sufficient for general evaluation of exposures to health care workers. The approach used to select the five agents was to obtain a list of the agents used most frequently in both a cancer hospital and an out patient cancer treatment center and then review the list to determine which agents were potentially more hazardous to human health. From these reviews, it was decided to attempt to develop an analytical method able to detect and quantify the presence of 5-fluorouracil, ifosfamide, cyclophosphamide, doxorubicin HCl. and paclitaxol.

A reverse-phase HPLC was selected for method development. The HPLC analytical method developed is able to detect all five agents of interest at minimum detectable concentrations of 0.5 ug/mL for each of the five agents.

BACKGROUND

A variety of antineoplastic agents are used in both hospital and outpatient cancer treatment centers. Many of these agents are potentially hazardous to the health of those health care workers involved in preparation and administration of these agents (e.g., oncology pharmacists and nurses),[1] as well as those involved in care of cancer patients (e.g.,

nurses, housekeepers, and janitors). It is therefore important to have accurate methods for evaluation of the exposures received by these individuals from such agents. This requires an accurate and sensitive analytical method to detect concentrations of such agents at very low concentrations.

Currently, acceptable analytical methods do exist for several of these antineoplastics, but usually only for an individual agent or for small groups of chemically similar agents.[2-14] However, this method was developed to provide analytical detection for a wider range of the types of agents use, and with a single analysis. This capability could, for example, provide more information on exposures to health care workers from such agents.

The reverse phase HPLC method is most often referred to in current literature on analytical methods for determination of antineoplastic agents.[2-14] This method appears to be most feasible for attaining the maximum sensitivity (lower limit of detection) when used for detection of multiple antineoplastics in both air and surface samples. As a result of this information, HPLC was selected for the method to be developed.

Thus, the major objective associated with this project was the enhancement of an existing HPLC method of analysis for cyclophosphamide to improve sensitivity and include accurate and sensitive detection and quantification for four other antineoplastic agents: ifosfamide, 5-fluorouracil, doxorubicin, and paclitaxel.

It was decided that the ability to simultaneously evaluate five agents was sufficient for general exposure evaluation. The approach used to decide which agents to include in this analytical method began by first obtaining a list of those agents most frequently used in both a cancer hospital and in an outpatient cancer treatment center. The

list of agents commonly used by both was then reviewed to determine which agents were potentially more hazardous to human health. From this review of agents it was decided to attempt to develop an analytical method able to acceptably detect and quantify the presence of cyclophosphamide, ifosfamide, 5-fluorouracil, doxorubicin HCl, and paclitaxol.

Many of these agents are cytostatic drugs that have pharmacological properties linked with potential genotoxicological hazards. Because the mechanisms of interaction of these drugs frequently involve interaction with deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or protein synthesis, many are known to have carcinogenic or mutagenic effects.[15-27]

Cyclophosphamide is an alkylating agent. It is classified by the International Agency for Research on Cancer (IARC) as both animal and human carcinogen, mutagen, and teratogen.[28-31] Cyclophosphamide is the drug of choice for the treatment of many types of cancer (e.g., Hodgkin's disease, multiple myeloma, a variety of leukemias, adenocarcinoma of the ovary, lymphomas, and breast cancer) and is an essential component of many effective drug combinations.[7,26-36]

Ifosfamide is a structural isomer of cyclophosphamide, but there are substantial differences in the spectrum of antitumor activity and toxicity between ifosfamide and cyclophosphamide.[27,37,38] Ifosfamide is also an alkylating agent and has been shown to be carcinogenic in rats, with female rats showing a significant incidence of leiomyosarcomas (a benign tumor) and mammary fibroadenomas. The mutagenic potential of ifosfamide has been documented in bacterial systems *in vitro* and mammalian cells *in vivo*.

5-Fluorouracil is a fluorinated pyrimidine and is an antimetabolite. It interferes with the synthesis of DNA and inhibits formation of RNA. The effects are most marked in those cells that grow more rapidly and which take up fluorouracil at a more rapid rate, such as in a fetus and a cancer. For this reason, 5-fluorouracil falls in Pregnancy category D, Teratogenic effects risk.[39,40]

Doxorubicin HCl is a cytotoxic anthracycline antibiotic isolated from cultures of the fungus *Streptomyces peucetius* var. *caesius*.[41] The most common uses for doxorubicin in cancer therapy is for various types of testicular cancer, leukemia, Ewing's sarcoma, Hodgkin's disease, and Kaposi's sarcoma.[42] Doxorubicin has been shown to have mutagenic and carcinogenic properties in experimental models.

Paclitaxel, which is a diterpene amide, is an antimicrotubule agent that was first isolated from the bark of the Pacific Yew (*Taxus brevifolia*) and is the primary component of Taxol, the commercially available agent.[43,44] Therapeutically, Taxol is of significant importance for treatment of ovarian cancer, breast cancer, carcinoma of the lungs, and head and neck carcinoma. The major toxicity is bone marrow depression with neutropenia. The appearance of this effect is the common dose-limiting toxicity. Taxol can cause fetal harm when administered to a pregnant woman.

Based on the health hazard information for the five agents of interest, an emphasis was placed on cyclophosphamide for developing an acceptable analytical method. This was because it is a known human carcinogen (IARC) and because of its high frequency of use and in relatively high concentrations.

A review of the physical characteristics of each antineoplastic of interest (fluorouracil, ifosfamide, cyclophosphamide, doxorubicin, and paclitaxol) was conducted

(see Table 1), followed by a review of existing analytical methods. Results from collection and review of this information found a significant difference in chemical structures, and chemical and physical characteristics between most agents, along with a variety of analytical methods having been developed for their individual detection. Methods included gas chromatography (GC),[2] gas chromatography-mass spectroscopy (GC-MS),[8] gas chromatography-tandem mass spectroscopy (GC-MS-MS),[3] and reverse-phase HPLC.[2,6,7,9-14] The most frequently used was the reverse-phase HPLC. Therefore, a reverse-phase HPLC system was chosen for analytical method development.

Table 1.
Physical Characteristics of Antineoplastics

Antineoplastic & Formula	Molecular Weight	Melting Point	Soluble In
5-Fluorouracil $C_4H_3FN_2O_2$	130.1	282 °C	Water
Ifosfamide $C_7H_{15}Cl_2N_2O_2P$	261.1	48 °C	Water, Saline or Methanol
Cyclophosphamide $C_7H_{15}Cl_2N_2O_2P \cdot H_2O$	279.1	45 °C	Water, Saline or Methanol
Doxorubicin HCl $C_{27}H_{29}NO_{11} \cdot HCl$	580.0	209 °C	Water
Paclitaxel $C_{47}H_{51}NO_{14}$	853.9	217 °C	Methanol (Highly Lipophilic)

From Manufacturers Data Sheets[2,7,8,10,11]

MATERIALS

Analytical equipment. The analytical equipment used for developing this method was a BIO-RAD MAPS 100 (Monoclonal Antibody Purification System) Preparative System, with a programmable ultraviolet-visible spectrophotometer (UV/VIS)

monitor. This is a reverse-phase HPLC unit equipped with a precision flow pump, an auto sampler with an injection loop of 500 µL, and a UV-VIS detector.

Because of an irreparable failure with the MAPS 100 HPLC unit, the stability tests were conducted on a newer BIORAD HRLC w/Model 1790 Programmable UV/VIS Monitor. This newer reverse-phase HPLC unit was also equipped with a precision flow pump, an auto sampler with an injection loop of 500 µL and a UV-VIS detector. Sensitivity (area under full scale [AUFS]) for this instrument was set at 0.05 for four of the agents, with 5-fluorouracil having an AUFS setting of 0.4. This instrument proved to have much greater sensitivity than the MAPS 100, thus the significant difference in the peak heights for the same agents was tested at the same concentrations.

UV detection. Of initial importance was determination of an acceptable wavelength setting for the UV detector. Because of the significant differences between most of the agents, it was important to assure a common UV range existed to assure simultaneous detection

In the literature, a number of different UV wavelengths have been used in past and existing analytical methods for the agents of interest. The wavelengths were 193 nm for cyclophosphamide,[13] 195 to 200 nm for cyclophosphamide and ifosfamide (Bristol Myers),[4] and 207 nm for ifosfamide (Zheng, 9-73)[37]; 214 nm to 254 nm, and 260 nm and 266 nm (after methanol extraction) for 5-fluorouracil;[40,45] 216 nm for doxorubicin;[42] and 230 nm for paclitaxol.[46] These are only a few of the different UV wavelength settings found in the literature search. Subsequently, a UV spectrum analysis using a Beckman DU-600 analyzer (spectrophotometer) was conducted on stock standards of each agent

(i.e., in methanol and buffered water). Because of the limited absorption of UV radiation by cyclophosphamide and ifosfamide above 210 nm, it was decided to use 195 nm for the optimum wavelength, where all five agents of interest indicated strong absorption.

HPLC column selection. Review of the literature found a variety of separation columns used in the analysis of antineoplastics. From among those, the Waters Symmetry C8, 3.5 μm, 4.6 mm x 150 mm inside dimensions (ID), column (Waters Part No. WAT200630) was chosen for use initially. However, this column was prone to fouling and also caused significant backpressure in the HPLC, even when using a 5.0 μm Symmetry guard column ahead of the column. After the initial evaluation of analytical range, this column was replaced with a Waters Symmetry C18, 5.0 μm, 4.6 mm x150 mm ID (Waters Part No. WAT045905). Both columns demonstrated acceptable separation of the components, but the C18 column was selected for all subsequent analyses on the HPLC because it was much less prone to fouling and did not cause exceptional backpressure.

Mobile phase. After reviewing mobile phase blends used in other HPLC analytical methods, it was decided to use an ACN:potassium phosphate buffer. A 10 mM potassium phosphate buffer was prepared by combining 1.1936 g of potassium phosphate, monobasic (KH_2PO_4) and 0.2143 g potassium phosphate, and dibasic (K_2HPO_4) with 1.0 L of Milli-Q water to form a pH 6.0 buffer. After testing, it was decided the optimum blend for assuring good separation and maintaining minimal time for the agents of interest was a 22.75% ACN:77.25% buffered water combination. It should be mentioned that all mobile phase blends were filtered through 0.2 μm filters prior to introduction into the

HPLC system. The mobile phase usually used for analysis of Taxol contains methanol. Chan et al.[36] used methanol:ACN:ammonium acetate ratio in a 20:25:55 mix.

Pump flow rate. Pump flow rate was also initially tested at 1.0 mL/minute and was used during the determination of analytical range for each agent of interest. However, because of the reduction in backpressure when the C18 column was installed, the optimum flow rate was changed to 1.2 mL/minute. An isocratic flow was used for 20 minutes, followed by a 30-minute gradient phase. The gradient phase began at 22.75% ACN and water and increasing to 70% ACN and water to increase the ability to detect paclitaxel (Taxol).[43,46] The following 5 minutes was used to reverse the gradient phase back to its original concentration of 22.75% acetonitrile, followed by 5 minutes of operation at the 22.75% isocratic phase to assure return to the a stable original baseline (60 minutes total run time).

Standard preparation. Stock standards were prepared in essentially the same fashion for each of the agents of interest. Those that were received in vials in dry form were first reconstituted in accordance with the manufacturer's recommendations. This was usually the addition of a specific quantity of distilled water. To minimize exposures during this reconstitution step, the vial septum was equipped with an air filter to allow for discharge of air as the vial was charged with distilled water.

All agents, those reconstituted to liquid form and those received in liquid form, were first blended with a 50:50 methanol:Milli-Q water blend. For example, cyclophosphamide was reconstituted to 20 mg/mL. An aliquot of 1.0 mL was withdrawn by syr-

inge and placed in a plastic vial (15 mL polypropylene, disposable centrifuge tubes with screw-on cap), followed by an aliquot of 9 mL of the methanol:water blend. This would form a 2.0-mg/mL stock standard. Subsequent cyclophosphamide standards were formed by dilutions of this standard with Milli-Q water. For example, a 1.0 mL aliquot of the 2.0-mg/mL stock standard and 9.0-mL Milli-Q water provided a 200 ug/mL standard.

Commercial products contained the pure agents (99.99% purity) and other agents with the exception of Taxol. Paclitaxel was tested in pure form due to the presence of other taxanes in the Taxol product, causing difficulty in identifying the peak of interest. The agents and respective products specifically evaluated are shown in Table 2 below.

Table 2.
Information on Agents Used to Prepare Standards

Brand Name	Composition	Lot No.	Manufacturer
Cytoxan (for injection)	Cyclophosphamide (20 mg/mL)	Lot 9G22823	Meade-Johnson (Bristol Myers Sq.)
Ifex (for injection)	Ifosphamide (50 mg/mL)	Lot KCS99	Meade-Johnson (Bristol Myers Sq.)
Doxorubicin HCl	Doxorubicin HCl (2 mg/mL)	Lot 123200A Lot 93592	Novaplus Bedford Labs
Adricil (for injection)	Fluorouracil (50 mg/mL)	Lot FFA221	Pharmacia & Upjohn
Paclitaxel (99.99%)	Paclitaxel	Not Available	Sigma

From these products, stock standards were made, which were further diluted to arrive at stock standards of varying concentrations for each of the five agents being studied.

Quality assurance was maintained by using all products prior to their manufacturer's expiration date. For this portion of the study, determining the acceptable analytical detection range and validating the method for simultaneous analysis of all five agents, the typical concentrations prepared were 1.0 µg/mL, 2.0 µg/mL, 5.0 µg/mL, and 10 µg/mL. Paclitaxel was validated at 2.0 µg/mL, 5.0 µg/mL, 10 µg/mL, and 20 µg/mL because of difficulty in avoiding precipitating out of the paclitaxel, possibly because of a reduction in methanol present, when further dilutions were attempted.

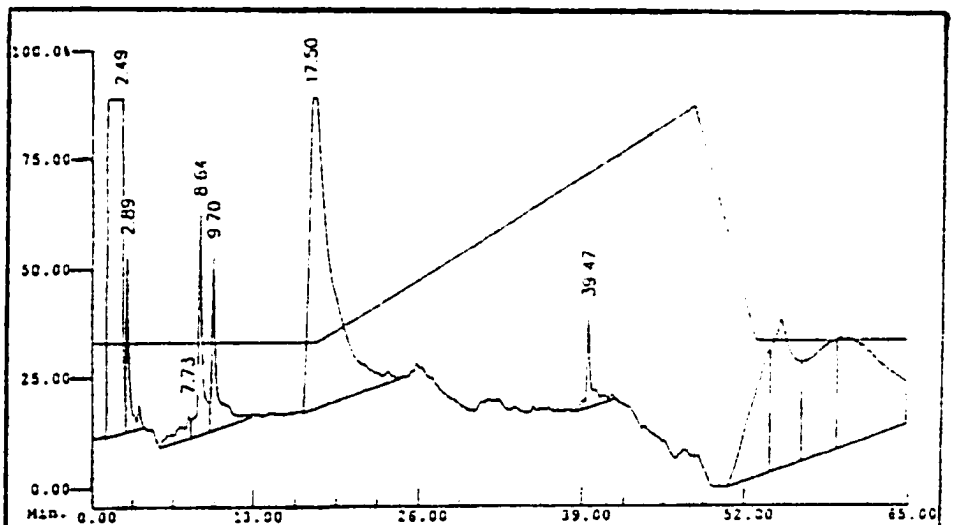
RESULTS

The typical peak separation and response obtained from using this analytical method on the newer HPLC system is shown in Figure 1 with an analysis using an AUFS of 0.05. It should be pointed out that an analysis conducted on the MAPS 100 with all agents, except paclitaxel, at 10-µg/mL, 5-fluorouracil appeared at 3.45 minutes, ifosfamide at 9.46 minutes, cyclophosphamide appeared at 10.62 minutes, and doxorubicin HCl appeared at 15.61 minutes. The peak for paclitaxel at 5.0 µg/mL appeared at 38.60 minutes or about the same time as the newer HPLC system after the initiation of the gradient phase to elute the paclitaxel.

The following tables (Tables 3 – 7) provide information used for developing the calibration curves for the various contaminants at the respective concentrations detected. The following calibration curves (Figures 2, 3, 4, and 5) indicate the linear slope and Y-intercept for average recoveries observed. The peak heights indicated for 5-fluorouracil, cyclophosphamide, ifosfamide, and doxorubicin HCl are all in computer units, which are in direct relation to absorption units. All peak heights were obtained by actual measure-

ment of the peak from the baseline. This was done instead of using the computer units for peak height from the HPLC analytical program because of the difference in recognizing the baseline from analysis to analysis. However, the analyses for paclitaxel did rely on the computer units for peak height due to a problem with the baseline disappearing from observation during the gradient portion in some analyses. Thus, the resulting peaks could not be manually measured, and computer determination of peak height was used.

Figure 1. Typical HPLC Chromatogram (Newer HPLC) for all five agents on a single run at AUFS of 0.05 (fluorouracil at 5 ug/mL; ifosfamide and cyclophosphamide at 10 ug/mL; doxorubicin at 20 ug/mL; and paclitaxel at 3 ug/mL).



Min.	Agent	Min.	Agent
2.49	Solvent Front	9.70	Cyclophosphamide
2.89	5-Fluorouracil	17.50	Doxorubicin HCl
8.64	Ifosfamide	39.47	Paclitaxel

Additionally, the standard curve determined from the average results for each agent at the various test concentrations was fitted with a trend line and also statistically analyzed to determine linearity. Analyses were conducted to develop a "standard curve"

for each of the antineoplastics of interest and to determine the linearity for each. Except for paclitaxel, results for each antineoplastic studied are discussed in the order it appears on the HPLC chromatogram.

Results from analyses conducted on 5-fluorouracil for concentrations from 0.5 to 5.0 ug/mL, which appears on the chromatogram at approximately 3 minutes, showed that the calibration curve was generally linear, with an R^2 of 0.9439 (see Table 3 and Figure 2). It should be noted that an R^2 is the variance of y that can be explained by the variable x. Thus if $R^2 = 1.00$, all variation in y can be explained by the variation in x, and all data points fall on the regression line.[47] However, at the concentration for 10.0 ug/mL, the HPLC detector response was nearly the same as the response for 5.0 ug/mL, indicating the linear portion of the line had been exceeded (Table 3 and Figure 3). This would indicate an upper limit for detection of 5-fluorouracil of approximately 5.0 ug/mL.

Paclitaxol was tested at concentrations ranging from 2.0 to 20.0 ug/mL and was found to appear on the gradient portion of the analysis at approximately 39 minutes. Although the R^2 was found to indicate minimal variance with concentration having an R^2 of 0.996, there was much variation in both the standard deviation (from 4.0 absorbance units [AUs] at 2.0 ug/mL to 145.5 AUs at 10.0 ug/mL) and coefficient of variation (from 3.7% to 19.7%) at the concentrations tested (see Table 4 and Figure 2).

Results from analyses conducted on ifosfamide and cyclophosphamide for concentrations ranging from 0.5 to 10.0 ug/mL, which appear on the chromatogram at approximately 8 and 9 minutes respectively, showed a minimal variance with R^2 values of 0.9906 and 0.9703, respectively. Accuracy and precision were also very good for ifos-

famide and cyclophosphamide with most standard deviations less than 1.0 AU and coefficients of variation less than 5.0% (see Tables 5 and 6 and Figures 4 and 5).

Doxorubicin HCl results for concentrations ranging from 0.5 to 5.0 ug/mL, which appears on the isocratic portion of the analysis at approximately 14 minutes, showed a minimal variation with an R^2 of 0.9883 (see Table 7 and Figure 4), but this deteriorated slightly with an R^2 of 0.9252 when analysis at 10.0 ug/mL was included (Figure 5). The standard deviation ranged from 0.06 to 2.45 AUs, and the coefficient of variation (C.V.) ranged from 2.0 % to 15.5% at the concentrations tested. This was much more variation in both the standard deviation and coefficient of variation at the concentrations tested than with the ifosfamide and cyclophosphamide. But the most noticeable concern for doxorubicin was in it being observed to deteriorate relatively rapidly when in contact with some of the other agents.

Table 3.
Analytical Results for 5-Fluorouracil to 10.0 μg/mL

	Concentration (ug/mL)				
Flourouracil	0.5	1.0	2.0	5.0	10.0
Average Peak Height (AUs):	0.0352	0.0713	0.1194	0.1965	0.1955
Number of Samples (*n*)	5	6	6	5	5
S.D. (AUs):	0.0010	0.0015	0.0002	0.0024	0.0032
C.V. (%)	2.98	2.12	0.19	1.23	1.97
Slope	0.019				
Y intercept	0.046				

Table 4.
Analytical Results for Paclitaxel to 20.0 µg/mL

	Concentration (ug/mL)			
Paclitaxel	2.0	5.0	10.0	20.0
Average Peak Height (AUs):	0.0462	0.2126	0.4929	0.9354
Number of Samples (*n*)	4	6	4	6
S.D. (AUs):	0.003	0.025	0.097	0.033
C.V. (%)	5.8	11.7	19.7	3.5
Slope	0.05			
Y intercept	0.71			

Table 5.
Analytical Results for Ifosfamide to 10.0 µg/mL

	Concentration (ug/mL)				
Ifosfamide	0.5	1.0	2.0	5.0	10.0
Average Peak Height (AUs):	0.0013	0.0022	0.0031	0.0093	0.0156
Number of Samples (*n*)	6	6	6	5	4
S.D. (AUs):	0.00004	0.00005	0.00006	0.00025	0.00066
C.V. (%)	3.31	2.31	2.06	2.69	4.23
Slope	0.0016				
Y intercept	0.0004				

Table 6.
Analytical Results for Cyclophosphamide to 10.0 µg/mL

	Concentration (ug/mL)				
Cyclophosphamide	0.5	1.0	2.0	5.0	10.0
Average Peak Height (AUs):	0.0013	0.0018	0.0029	0.0092	0.0135
Number of Samples (n)	6	6	6	6	4
S.D. (AUs):	0.00005	0.00006	0.00004	.0005	0.0012
C.V. (%)	3.62	3.15	1.26	5.76	9.10
Slope	0.0014				
Y intercept	0.0005				

Table 7.
Analytical Results for Doxorubicin HCl to 10.0-µg/mL

	Concentration (ug/mL)				
Doxorubicin HCl	0.5	1.0	2.0	5.0	10.0
Average Peak Height (AUs):	.0029	.0046	.0086	.0286	.0356
Number of Samples (*n*)	6	6	6	6	4
S.D. (AUs):	0.0001	0.001	0.001	0.002	0.002
C.V. (%)	2.04	18.83	6.72	6.96	6.90
Slope	0.0036				
Y intercept	0.0026				

Figure 2. Standard curve for 5-fluorouracil based on average recovery at each concentration to 5.0 ug/mL and paclitaxel to 20.0 ug/mL.

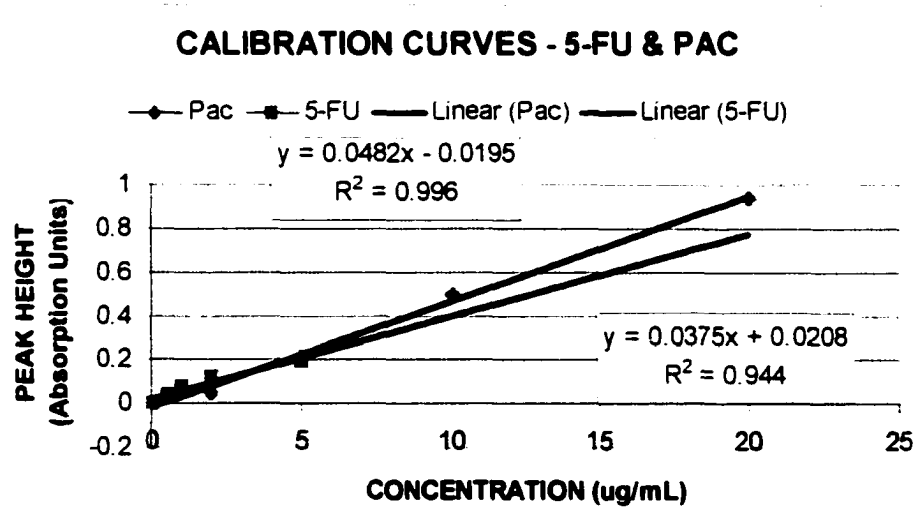


Figure 3. Standard curve for 5-fluorouracil based on average recovery at each concentration up to 10.0 ug/mL and paclitaxel to 20.0 ug/mL.

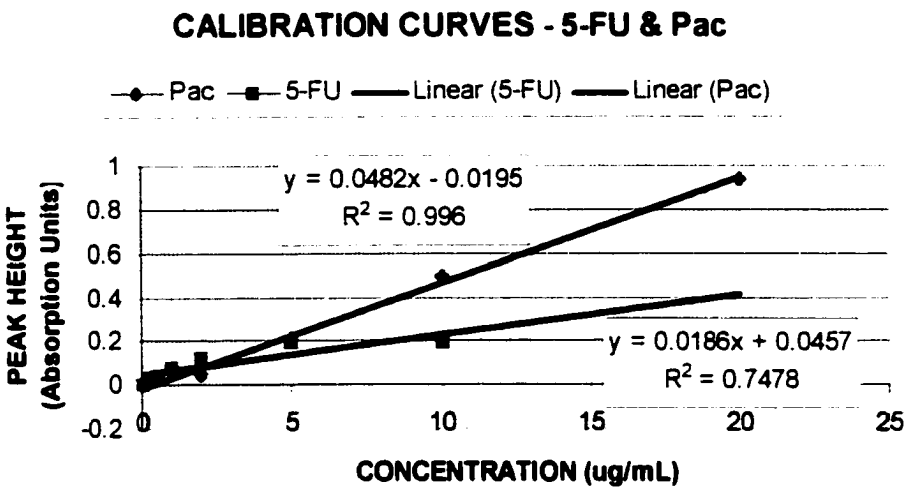


Figure 4. Standard curves for ifosfamide, cyclophosphamide, and doxorubicin HCl based on average recovery at each concentration up to 5.0 ug/mL.

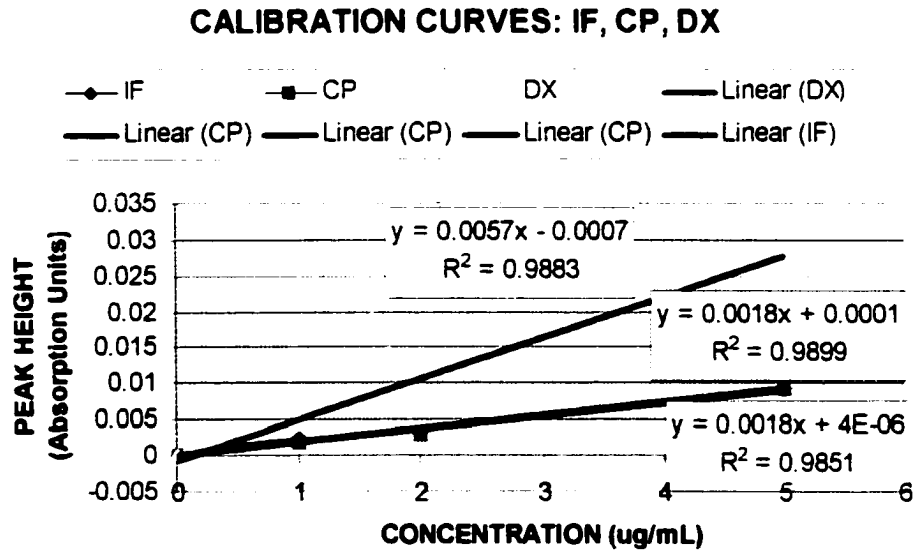
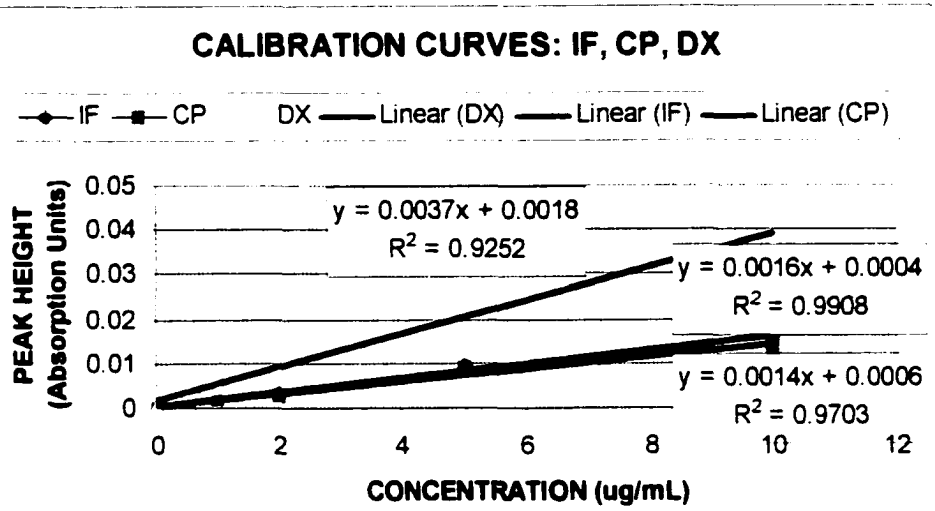


Figure 5. Standard curves for ifosfamide, cyclophosphamide, and doxorubicin HCl based on average recovery at each concentration up to 10.0 ug/mL.



STABILITY TESTING

These stability tests were conducted on each agent using standard solutions of 10.0 µg/mL for 5-fluorouracil, ifosfamide, cyclophosphamide, and doxorubicin HCl. A 3.0 ug/mL solution was used for paclitaxel because of its increased response to the UV wavelength setting. The number of analytical results for sample stability ranged from two to four for each agent for each day indicated.

Each agent was initially prepared as a stock standard using a 50:50 blend of methanol-buffered water (6.0 pH) and then diluted with Milli-Q water to the appropriate concentration for test purposes. These solutions were maintained at room (ambient) temperatures and analyzed on the days indicated for each. The results for each antineoplastic studied are discussed in the order it appears on the HPLC chromatogram. It should be noted that analyses were attempted at additional times than those shown in the tables be-

low, but because of difficulties with the HPLC, either with pressure drops in the mobile phase or detector problems, these results were not able to be used.

As indicated in the Results section above, 5-fluorouracil is the first agent to appear on the chromatogram after the solvent peak (about 3 minutes). Analyses were conducted on the day prepared (zero or 0 day) and days 6, 8, 17, 27, and 31. These results indicated an approximate deterioration of 20% in the first week, and approximately 41% over a 31-day period (Figure 6). Paclitaxol was tested at 0 day and days 5 and 6. Only a small fraction of deterioration was observed over that time period (Figure 6).

Ifosfamide and cyclophosphamide were tested at 0, 1, 2, and 14 days. The recovery at day 2 indicates an average deterioration of 13% after 2 days and a 32% deterioration after 14 days for ifosfamide and no detectable deterioration at day 2 but a 33%

Figure 6. Stability test results for 5-fluorouracil based on testing at a 10.0 ug/mL concentration over a period of 31 days and paclitaxel based on testing at 3.0 ug/ml concentration over a period of 7 days. The number of paclitaxel stability samples analyzed per day indicated are 3 for day 0, 1 for day 5, and 3 for day 6; number of fluorouracil samples analyzed per day indicated are 1 for day 0, 3 for day 6, 1 for day 8, 4 for day 17, 3 for day 27, and 2 for day 31.

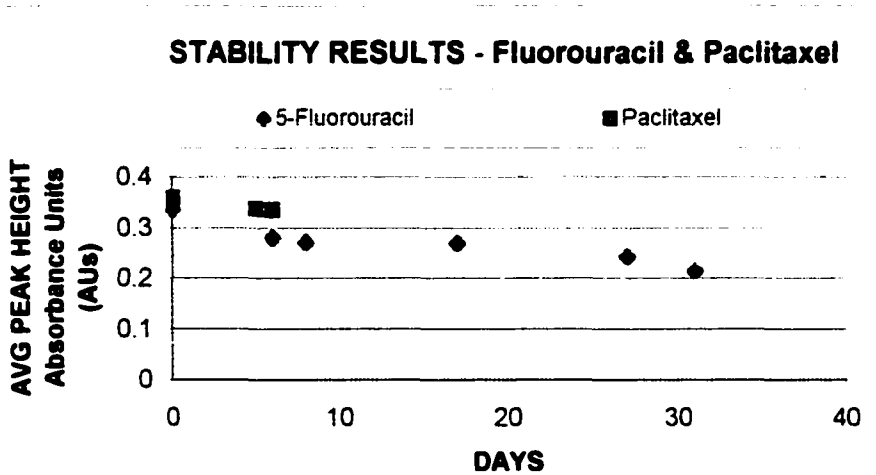
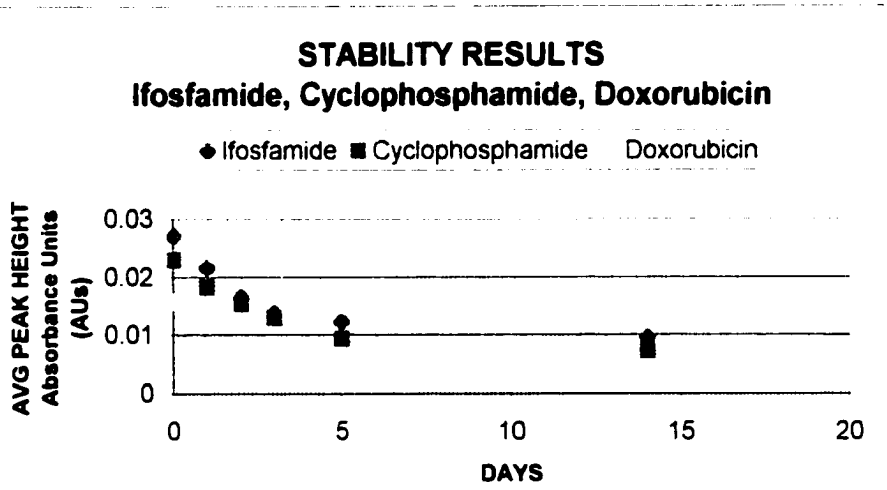


Figure 7. Stability test results based on testing of 10.0 ug/mL solutions of ifosfamide and cyclophosphamide over a period of 14 days and 5.0 ug/mL solution of doxorubicin over 17 days. Two samples ($N = 2$) were analyzed per day indicated for determination of stability for each agent (ifosfamide, cyclophosphamide, and doxorubicin HCl).



deterioration after 14 days for cyclophosphamide (Figure 7). Doxorubicin HCl was tested at 0, 6, and 17 days. The average deterioration was approximately 45% at day 6 and approximately 75% on day 17 (Figure 7).

DISCUSSION

Of primary importance for this study was the determination of an acceptable UV wavelength for the simultaneous detection of the five agents of interest. Settings of the UV wavelength for detection of cyclophosphamide in various reported studies have ranged from 193 nm to 270 nm.[12,13,43,48] An analysis by a spectrophotometer provided the information needed to determine an optimum UV wavelength (195 nm) for this analytical method. For information purposes, a wavelength of 254 nm was tested one-time

during this study because of it's appearance in some of the literature, but, as expected, it was not found to be a usable wavelength for this test.

Results from the tests on 5-fluorouracil indicated that it could be detected separate from the solvent peak on the chromatogram at concentrations of approximately 1.0 ug/mL or less. But for higher concentrations the analysis had to be reconducted with a higher AUFS setting to separate the 5-fluorouracil from the solvent peak for both identification and quantification.

Cyclophosphamide, the agent of most interest in this study because of its frequency of use and its being recognized as a human carcinogen, and ifosfamide, a similar type of agent, were found to be very accurately measured to levels of 0.5 ug/mL. Based on the increased sensitivity observed when the newer HPLC was used in the stability testing, it is expected that the lower minimum detection limit is likely to be 0.1 to 0.2 ug/mL. This would make this method competitive with use of the HPLC-MS systems for acceptable low-level detection capabilities but without the derivatisation step and higher equipment and time costs.

Both standard curve and stability test results for doxorubicin HCl indicated it to be more stable than ifosfamide and cyclophosphamide, but this was for the pure agent. It was noted that, when doxorubicin HCl is blended with some other agents or comes in contact with surfaces containing various organic chemicals, the deterioration can be very rapid with as much as a 30% deterioration in one day.

Some difficulty was observed in making paclitaxel standards. It was found that paclitaxel is less soluble in solvents other than methanol and that a precipitate would form or the paclitaxel would not completely dissolve in some blends of methanol, ACN,

and water. This caused some difficulty in the development of a standard curve and a stability analysis. Although both were accomplished with this method, the results did indicate a relatively wide fluctuation in results for analysis of the agent at the same concentration (high standard deviation and coefficient of variation).

CONCLUSIONS

Overall, results of this study found the analytical method described above to be acceptable for the detection and identification of all five agents of interest in a single analysis. The lower limit of detection, based on results from using the MAPS 100 HPLC, was 0.5 ug/mL for four of the agents: 5-fluorouracil, ifosfamide, cyclophosphamide, and doxorubicin HCl. Paclitaxel's lower limit of detection was determined to be 2.0 ug/mL on this unit. Quantification of these agents is also acceptable for these agents, except that it may be necessary to reconduct the analysis with a higher AUFS for the purposes of quantifying 5-fluorouracil.

The stability results did indicate varying degrees of deterioration when stored at ambient temperatures. This information supports storing standards at -20 °C and that both standards and samples should be assayed as soon as possible after the samples are collected to avoid error due to loss of potency (deterioration). Ideally, fresh stock standards should be prepared for each new day of analyzing for these agents.

Because of this method was found to be an effective tool for the simultaneous analysis of five different antineoplastics, it provides the capability to conduct a more comprehensive evaluation of facilities handling antineoplastic agents. For example, analysis can be conducted on samples collected off various types of surfaces commonly

found in hospitals and pharmacys or on solid sorbents used to collect air contaminant samples. Data from such samples can provide valuable information on exposure potential to individuals working in such facilities, both the potential for exposure via skin contact with contaminated surfaces and exposure to contaminated air within their work areas.

Additionally, this method provides the ability to conduct acceptable risk analysis of individuals working in areas where these agents may be handled. This is very important because of the need for more accurate information to associate exposure information to actual dose concentrations (e.g., uptake in the body) of these agents detected in the urine of oncology pharmacists and nurses. [8,49,50]

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DEVELOPMENT AND REVIEW OF A SURFACE MONITORING METHOD FOR SELECT ANTINEOPLASTICS

by

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ABSTRACT

An important aspect of conducting a hazard analysis in areas where antineoplastics are handled is the assessment for surface contamination. A number of methods already exist, but some of these methods are cumbersome or may lack adequate sensitivity to be of much use. This study involved the evaluation of two different surface wipe materials, Whatman Grade #42, 55 mm diameter circle filters and Kimwipe EX-L precision wipes. These wipe materials were tested on three different types of surfaces commonly found in areas where antineoplastic agents are handled: vinyl floor tile, resin type counter surfaces, and stainless steel. In addition, a blend of methanol, acetonitrile, and buffered water were used for both the wetting agent to wipe the surfaces, and as the desorbing solution. Agents evaluated were fluorouracil, ifosphamide, cyclophosphamide, doxorubicin, and paclitaxel. Cyclophosphamide was of most interest because it was identified as a human carcinogen. Results of this study found that use of the Whatman #42, 55 mm circle filters, which involves use of two circles used in sequence but desorbed together for the first wipe, was the best for surface testing, especially for ifosphamide and cyclophosphamide.

INTRODUCTION

Biological evidence of absorption through the skin exists for several antineoplastic agents. For example, M. Hirst[1] detected cyclophosphamide in the urine of two nurses who handled the agent, thereby documenting worker absorption. Hirst also documented skin absorption in human volunteers by using gas chromatography (GC) after topical application of the drug. Actually, it has been reported that dermal and ingestive routes of

entry are more significant than inhalation for a large number of these agents.[2] Many of these agents are cytostatic drugs having pharmacological properties linked with potential genotoxicological hazards. Because the mechanisms of interaction of these drugs frequently involve interaction with deoxyribonucleic acid (DNA), ribonucleic acid (RNA) or protein synthesis, many are known to have carcinogenic or mutagenic effects.[3-7]

Thus, when conducting a worksite hazard analysis at locations where antineoplastics agents are used, it is important to assess the presence of surface contamination. Many of these agents are absorbed through the skin, making skin contact with contaminated surfaces a likely source of exposure. Therefore, it is as important to identify and quantify the presence of antineoplastics on various surfaces available for skin contact as it is for the evaluation of contaminants in the air.

A common method for evaluation of surface contamination for identifying the presence of potentially hazardous agents is "wipe sampling."[8-14] This method is also used to evaluate the effectiveness of personal protective equipment, housekeeping, and decontamination programs. Terms like "swipe sampling" and "smear sampling" are synonymous with wipe sampling, and they all describe the techniques used to assess surface contamination, whether it is on work surfaces, personal protective equipment (PPE) surfaces, or skin. This document will use the term wipe sampling for evaluation of surface contamination.

OBJECTIVE OF STUDY

The objective of this study was to identify an acceptable method for evaluation of surfaces for contamination from antineoplastic agents. The method needed to be able to

identify an effective material for wiping surfaces of various types; have acceptable absorption and desorption capabilities, especially for cyclophosphamide; and have sufficient sensitivity to detect the agent of interest at low enough concentrations to be meaningful. This study was conducted with these objectives as a primary consideration.

MATERIALS

Agents. Trade name commercial products were used for evaluation of each antineoplastic except for paclitaxel. The trade name product for paclitaxel therapy is Taxol (Bristol Myers Squibb), and it was found that there were interferences in separation of known and unknown contaminants in Taxol. Table 1 provides the information on the agents used in this study.

Table 1.
Information on Agents Used to Make Standards

Brand Name	Composition	Lot No.	Manufacturer
Cytoxan (for injection)	Cyclophosphamide (20 mg/mL)	Lot 9G22823	Meade-Johnson (Bristol Myers Sq.)
Ifex (for injection)	Ifosphamide (50 mg/mL)	Lot KCS99	Meade-Johnson (Bristol Myers Sq.)
Doxorubicin HCl	Doxorubicin HCl (2 mg/mL)	Lot 123200A Lot 93592	Novaplus Bedford Labs
Adricil (for injection)	Fluorouracil (50 mg/mL)	Lot FFA221	Pharmacia & Upjohn
Paclitaxel (99.99%)	Paclitaxel	Not Available	Sigma

Each of these five agents was first reconstituted with distilled water as applicable and in accordance with manufacturer's instructions and then prepared into stock solutions by dilution in a blend of 50% methanol and 50% Milli-Q water. Any subsequent dilutions to prepare standards of a desired concentration were obtained by blending with Milli-Q water.

Selectivity (separation of peaks) was carried out by reference to calibration curves constructed from the analysis of freshly prepared reference standards. These reference standards were prepared from the stock solutions of each agent and varied from containing a single agent at a specific concentration to containing all five agents at various concentrations. The selectivity of the method was verified by the analysis of blank and spiked samples.

Selection of Surfaces for Method Evaluation. It was determined that the most common surfaces found in pharmacys and oncology clinics are stainless steel as in the interior of biosafety cabinets; resin surfaces from counter tops in pharmacys, lab and therapy areas; and vinyl flooring (12 inch x 12 inch squares). Portions of each type of surface were obtained: 2000-cm^2 sheets of stainless steel, 2400-cm^2 sections of resin counter surface, and 12 inch x12 inch (929 cm^2) vinyl flooring squares. Each type of surface to be tested for contamination recovery was marked to indicate areas of 600 cm^2 for this study.

Initial dilutions of the standards for the individual antineoplastic agent of interest to dose test surfaces were prepared at 400 µg/mL for 5-fluorouracil, ifosphamide, cyclophosphamide, and doxorubicin HCl. Initial dilutions prepared for paclitaxel was 120

µg/mL for the Whatman wipe testing and 200 µg/mL for the Kimwipe wipe testing. From these dilutions, 0.1 mL of each was removed from the respective dilution vials by a 1.0 mL syringe and spread evenly over the 600 cm^2 areas marked on each type of surface. This yielded an equivalent of 40 µg for each of the first four agents of interest and an equivalent of 20 µg and 12 µg for paclitaxel. Also, at the same time as treating the surfaces with the agents, a wipe material was spiked with the same quantity of each agent as being spread on the test surfaces. These spiked wipe materials served as analytical controls. After wiping and desorbing wipe material in 4.0 mL of desorbing solvent, this would be expected to yield a 10 µg/mL result for 5-fluorouracil, ifosphamide, cyclophosphamide, and doxorubicin HCl and 3.0 µg/mL of paclitaxel. This was assuming 100% recovery for each agent from each surface sample.

Selection of wipe materials. The next step was to determine the material to be used for conducting surface wipes. A variety of methods are available for collection of surface samples, but they can vary in a number of different parameters. For example, they may vary in the total surface area to wipe, the size and type of wipe sampling material, the type of solution used for wetting the wipe sampling material, and the volume of solution for desorption of the sample material.

After reviewing the existing methods, it was decided that two different materials would be used to identify the optimum for this method. The first material chosen was a Whatman Grade #42, which is an ashless 55 mm diameter cellulose circle filter. These were chosen after reviewing the composition of a variety of such filters/circle wipes,[16] observing the Whatman #1 Qualitative, 5.5 cm (55 mm) diameter circle filters had previ-

ously been used for some surface monitoring methods,[15] and after having a discussion with Whatman technical services. The second wipe material was a Kimwipe-EX-L Delicate Task Wipe, which is a rectangular 11.4 cm x 21.6 cm (4.5 inch x 8.5 inch) analytical wipe sheet. This material was manufactured by Kimberly Clark and was recommended for testing by one of their Customer Solutions (technical) representatives based on the composition (100% virgin wood fiber or nearly all cellulose) and good extractable performance.[17]

One question regarding the use of wipe materials was being able to assure the wipes were of uniform size and weight for assuring uniformity in both the ability to absorb contaminants and in the ability for desorbing of contaminants. The Whatman #42 filters are known to have acceptable consistency, but information was not readily available to determine the same for the Kimwipe EX-L; therefore, a test was conducted to determine consistency in the Kimwipes. Five wipes were initially removed from a new box of Kimwipes and discarded, and then 10 tissues were consecutively removed and individually weighed on a precision balance (Mettler 5-place balance). The average weight was found to be 466.7 mg, with a standard deviation of 9.8 mg and a coefficient of variation (C.V.) of 2.1%. This C.V. was within the range of 5.0%, which is usually considered acceptable for weight consistency. Therefore, Kimwipe EX-L was included as the second material for evaluation.

Development of a desorption solution. The next step in the development of the surface monitoring method was determination of the most effective desorption solution. Initially and based on existing methods, desorption solutions of methanol and water were

evaluated. Desorption solutions of 50:50% methanol:water, 60:40% methanol:water, and 70:30% methanol:water were evaluated. Desorption for ifosphamide was moderate (~70%), but desorption of cyclophosphamide was low (~50%) when using this mixture. Therefore, it was decided to evaluate a solution of 50:50% acetonitrile (ACN):water. Results of this were less for ifosphamide and about the same for cyclophosphamide (~50% for each), but response was much better for doxorubicin, fluorouracil, and paclitaxel (near 100% average recovery for each). Because of the variation in physical characteristics of the agents of interest in this study, especially polar aspects of each, and the differences in polarity of the solvent being considered for use, it was decided to test mixtures containing both solvents. After tests on various blends, it was determined that the most effective desorbent was a blend of 10% acetonitrile, 25% methanol, and 65% Milli-Q water, with the water buffered to pH 6.0 by blending 10 mM monobasic with dibasic potassium phosphate.[18] The buffer to pH 6.0 was chosen based on the pH used for other collection and analysis methods for cyclophosphamide already in use.[15,18] This blend provided optimum desorption of nearly 100% for cyclophosphamide, ifosphamide, 5-fluorouracil, and paclitaxel, with desorption of ~60% for doxorubicin HCl.

Surface sampling procedures. After each surface area had been treated with all five antineoplastics agents of interest, they were allowed to dry. Drying was accomplished in a laboratory illuminated with fluorescent lighting, humidity controlled to 60%, and temperature at 72 °F. Drying was usually allowed to occur over a period of 4 hours minimum. After donning latex gloves to avoid skin contact, testing was then conducted

as follows for the two different wipe materials, Whatman circle filters and Kimwipe sheets.

The first step was to evaluate the Whatman filters by spreading 0.5 mL of the desorbing solvent over the 600-cm^2 area of the surface to be wiped. This area was then wiped with a single Whatman #42 circle filter. The circle was then placed in a disposable petri plate to dry. This step was repeated with a second Whatman circle, which was also placed in a separate disposable petri plate to dry. Like the surfaces after dosing with the agents, drying of the wipe sampling material was accomplished in a laboratory with humidity controlled to 60% and temperature at 72 °F. When both were dried (4 hours minimum), they were placed together in a single covered 55-mm petri plate. Four milliliters of desorbing solution was injected onto the surface of the two circle filters. The desorption solvent was the same as that used in collection of the surface samples: a mixture of 10% ACN, 25% methanol, and 65% Milli-Q water buffered to pH 6.0. After the solvent was added, the closed plates were place on an orbital shaker (Lab-line orbital shaker) and shaken for 30 minutes at 100 rpm. After shaking, the desorbate was removed via a syringe equipped with a syringe filter (a Gelman 25 mm Acrodisc LC PVDF Syringe Filter, 0.2 um). This filtration was necessary for both the Whatman and Kimwipe wipe materials to remove fiber contaminants so they would not affect (e.g., plug) the separation column during analysis. Samples of standards were also filtered and analyzed for comparison to filtered desorbate to evaluate if any chemical interference from the filtering process might be present. No interferences were identified due to filtration. This filtered desorbate was then placed into a 15-mL disposable tube with a screw-on cap (Corning disposable non pyrogenic polypropylene centrifuge tubes with flat cap,) until it

could be analyzed. Subsequent wipes with a Whatman circle filter over the same surface area, usually three additional wipes, were conducted to better determine overall effectiveness of removal/recovery of the surface contamination. All subsequent wipes were individually desorbed. After desorbing, the desorbate was analyzed by the analytical method described in the analytical section below.

The next step was to evaluate the Kimwipes. These sheets were used similar to the Whatman circle filters, except a larger volume (1.5 mL) of desorbing solvent was spread across the area to be wiped, and only a single Kimwipe was used for the first, as well as subsequent, wipe testing. After wiping, the Kimwipes were allowed to dry, folded, and placed in a clear 10 mL glass Microvial with a screw-on cap and septum (from Kimble Glass, Inc.). Desorbing was accomplished by injecting 4.0 mL of the desorbing solvent described above into the vial. After the solvent was added, the vial(s) were placed on a orbital shaker (Lab-line orbital shaker) and shaken for 30 minutes at 150 rpm. After shaking, the desorbate was removed via a syringe equipped with a Whatman 13 mm Syringe Filter, 0.2 um (PVDF Filter Media, sterile and non pyrogenic) and placed into a 15 mL disposable tube with a screw-on cap. After desorbing, analysis of the desorbate was conducted by the same analytical method as used for the Whatman filter desorbate.

Analysis of the drugs

Analytical equipment. The analytical method used a Bio-Rad reversed-phase high performance liquid chromatograph (HPLC) equipped with a model 1790 programmable ultraviolet-visible spectrophotometer (UV/VIS) monitor. The system utilized a

500-µL injection loop and a Waters 150 x 4.6 mm, Symmetry C18, with 5.0-µm particles. The mobile phase for isocratic flow was 22.75% ACN in potassium phosphate buffered water having a pH of 6.0, and the flow rate was set at 1.2 mL/minute in all cases.

Determination of UV wavelength for detector. Each of the agents was scanned by spectrophotometry (Beckman DU-600) to determine their individual UV wavelength ranges in nanometers (nm) for UV detection. It was learned that the optimum UV range able to detect all five agents with the same analysis was between 190 and 200 nm (see Table 2). Thus, a UV wavelength of 195 nm was used.

Table 2.
Ultraviolet (UV) Detection Range for the Agents of Interest

Agent	UV Wavelength (nm)
Fluorouracil	195 to 210
Ifosphamide	190 to 195
Cyclophosphamide	190 to 195
Doxorubicin	190 to 195 and 230 to 235
Paclitaxel	190 to 210

Mobile phase. A combination isocratic and gradient method was found acceptable for detection of all five agents with a single analysis. The mobile phase was 22.75% ACN in Milli-Q water, with the water buffered by potassium phosphate to a pH 6.0 and filtered through a 0.20 µm filter under vacuum. Analysis was conducted in isocratic

phase for the first 20 minutes, followed by a 25 minute gradient phase to 70% ACN and buffered water. At the end of the gradient phase, the analysis was returned to isocratic at 22.75% ACN over another 5 minutes and a 10 minute final period to completely return to the normal baseline. Gas formation from the ACN was minimized by first degassing the any solutions containing ACN.

RESULTS

Surface monitoring results in percent recovery from tests of each of the five agents using Whatman filters as wipes are indicated in Table 3 for vinyl floor tiles, Table 4 for resin surfaces, and Tables 5 for stainless steel surfaces. Table 6 indicates the results in percent recovery for tests of each of the five agents recovered using Kimwipe EX-L wipes on stainless steel surfaces.

Detection of cyclophosphamide, which was the agent of most interest in this study, using Whatman circle filters, was found to have acceptable (vinyl floor tile) to very good (resin and stainless steel) average recovery and standard deviation (S.D.) from both the first set of wipes and after four wipes for each surface type, as shown in Table 7.

Very similar results were seen from evaluation of the ifosphamide wipe tests, indicating very good precision and accuracy for the method to detect agents on both resin and stainless steel surfaces. Acceptable precision and accuracy was observed for these agents on vinyl tile, with the differences believed to be due to the presence of a variety of other chemicals in the composition of the floor tiles and the wax surface treatment. Precision and accuracy were not as high for this method in recovery of the other three agents on the various surfaces.

Fluorouracil results were fully evaluated for resin surfaces and partially for stainless steel surfaces, but not on vinyl floor tile. This was due in part to the need to change the sensitivity (area under full scale [AUFS]) settings on the HPLC and reconduct the analyses to evaluate the chromatographic peak. At the AUFS settings used for the other agents, the fluorouracil peak was hidden in the solvent peak at the front of the chromatogram.

Doxorubicin was detected on each surface, but recovery with the first wipe was generally poor, generally less than 40%. Overall recovery was also generally poor. Paclitaxel could also be detected, but the results varied significantly and were generally less than 50% recovery for the first set of Whatman circle filter wipes.

DISCUSSION

Minimum concentration of detection. The preliminary goal for this method was to be able to attain a minimum concentration of detection for each of the agents of interest of at least 10 ng/cm^2, which is an equivalent to 1.0 µg/100 cm^2 (100 cm^2 is the size area typically wiped for detection of antineoplastic agents in other studies). The results in Table 8 indicate the minimum concentration of detection obtained using the Whatman #42 circle filters ranging from 0.2 to 4.0 ng/cm^2 (equivalent to 0.02 to 0.4 µg/100 cm^2), which is very acceptable for the evaluation of surface contamination on the types of surfaces tested. The precision and accuracy, based on standard deviation, were found to be generally very good for resin and stainless steel surfaces (S.D. typically ≤1.2%) and acceptable for vinyl floor tiles (S.D 7.3% for ifosfamide and cyclophosphamide).

Table 3.
Antineoplastic Recovery on Vinyl Tile Using Whatman Wipes

Spike Agent	(ug)	Average Recovery % (S.D.) Wipe 1	Wipe 2	Wipe 3	Wipe 4	Total
5-Fluorouracil	100	[a]	[a]	[a]	[a]	[a]
Ifosphamide	100	56.7 (7.3)	14.0 (1.7)	13.0 (2.8)	6.5 (1.5)	90.2 (12.5)
Cyclophosphamide	100	57.2 (7.3)	13.3 (1.7)	10.9 (2.8)	6.3 (1.5)	87.7 (15.3)
Doxorubicin HCl	100	39.6(14.0)	15.6 (2.7)	10.4 (4.5)	6.8 (1.8)	72.4 (17.6)
Paclitaxel	12	49.2 (0.7)	27.1 (0.8)	13.5 (1.1)	5.6 (0.5)	95.3 (2.9)

a. Fluorouracil peak hidden in solvent peak at front of the high performance liquid chomatogram.
Above test was repeated on six vinyl floor surfaces.

Table 4.
Antineoplastic Recovery on Resin Surfaces Using Whatman Wipes

Spike Agent	(ug)	Average Recovery % (S.D.) Wipe 1	Wipe 2	Wipe 3	Wipe 4	Total
5-Fluorouracil	40	48.2 (0.6)	29.1 (1.9)	19.8 (1.3)	<10.0	97.0 (5.1)
Ifosphamide	40	88.2 (0.7)	11.9 (0.4)	4.7 (0.1)	< 3.8	104.7 (0.8)
Cyclophosphamide	40	88.0 (1.0)	8.3 (0.6)	2.9 (0.2)	< 2.5	99.2 (0.5)
Doxorubicin HCl	40	50.2 (7.4)	38.4 (2.0)	3.0 (2.0)	N.A.	91.5 (5.4)
Paclitaxel	12	53.1 (8.7)	46.4 (8.7)	13.4 (1.9)	8.0 (1.2)	95.3 (2.9)

N.A. = No wipe performed.
Above test was repeated on six resin counter surfaces.

Table 5.
Antineoplastic Recovery on Stainless Steel Using Whatman Wipes

Spike Agent	(ug)	Average Recovery % (S.D.) Wipe 1	Wipe 2	Wipe 3	Wipe 4	Total
5-Fluorouracil	50	66.7 (0.0)	13.4 (1.8)	7.0 (0.6)	<4.0	87.0 (1.6)
Ifosphamide	40	78.0 (0.5)	9.8 (1.4)	7.6 (0.2)	<1.3	95.3 (1.7)
Cyclophosphamide	40	76.8 (1.2)	8.6 (0.4)	5.4 (1.0)	<1.3	90.8 (1.7)
Doxorubicin HCl	40	42.2 (1.5)	34.8 (1.4)	<5.0	N.A.	77.0 (0.5)
Paclitaxel	12	38.2 (0.6)	21.0 (0.6)	10.4 (0.9)	4.3 (0.4)	73.9 (2.3)

N.A. = No Wipe Performed.
Above test was repeated on six stainless steel surfaces.

Table 6.
Antineoplastic Recovery on Stainless Steel Using Kimwipe EX-L Wipes

Spike Agent	(ug)	Average Recovery % (S.D.) Wipe 1	Wipe 2	Wipe 3	Wipe 4	Total
5-Fluorouracil	50	[a]	[a]	16.6 (1.1)	7.6 (0.9)	[c]
Ifosphamide	40	112.4 (0.9)	95.0 (1.1)	82.4 (3.5)	83.0 (3.6)	372.8 (8.0)[b]
Cyclophosphamide	40	62.4 (8.3)	13.6 (0.6)	7.6 (0.4)	< 1.25	83.6 (7.4)
Doxorubicin HCl	40	4.2 (1.7)	6.4 (0.3)	3.0 (1.1)	N.A.[c]	18.2 (1.4)
Paclitaxel	20	29.8 (0.5)	30.7 (0.8)	18.0 (2.0)	< 5.0	85.9 (3.1)

a. Fluorouracil peak hidden in solvent peak at front of high performance liquid chromatogram.
b. Ifosphamide total recovery was significantly higher than spike due to con founding chemical in Kimwipe EX-L.
c. Not applicable due to wipe or wipes not performed.
Above test was repeated on six stainless steel surfaces.

Table 7.
Average Percent Recovery and Standard Deviation for Cyclophosphamide on Different Surfaces Using Whatman #42 Circle Filters

Surface Type	Avg Recovery (After first Wipe)	Standard Deviation	Avg Recovery (After four Wipes)	Standard Deviation
Vinyl Floor Tile	57.2%	7.3%	87.7%	15.3%
Resin Counter	88.0%	1.0%	99.2%	0.5%
Stainless Steel	76.8%	1.2%	90.8%	1.7%

Table 8.
Minimum Concentration of Detection for Each of the Five Agents of Interest on the Different Types of Surfaces Tested Using Whatman Circle Filters

Surface Type	Minimum (ng/cm^2)
5-Fluorouracil	
Vinyl Tile	Not Analyzed
Resin Surface	4.0
Stainless Steel	2.0
Ifosfamide	
Vinyl Tile	3.3
Resin Surface	2.5
Stainless Steel	0.8
Cyclophosphamide	
Vinyl Tile	3.3
Resin Surface	1.7
Stainless Steel	0.8
Doxorubicin HCl	
Vinyl Tile	2.0
Resin Surface	2.0
Stainless Steel	2.0
Paclitaxel	
Vinyl Tile	0.2
Resin Surface	0.3
Stainless Steel	0.2

Interpretation of results. The following paragraphs discuss the sensitivity and recovery of agents from the different surfaces tested and compares this new surface monitoring method to some of the other surface monitoring methods in use.

The method used to determin fluorouracil required reanalysis of the sample desorbate with a reduction in instrument sensitivity. This reduction in sensitivity is also likely to be the reason for the increase in the minimum concentration of detection and lower overall recovery as compared to the other agents on the same type surface. Sensitivity and recovery were excellent for ifosphamide and cyclophosphamide, based on total recovery (sum of four wipe analyses) being greater than 85% (generally >90%) for each agent on all three surfaces tested.

Doxorubicin was the lowest average recovery, but this is believed to be due, at least in part, to decomposition of the doxorubicin on the test surfaces. In the presence of acid (the standards were in a buffered solution of 6.0 pH) the glycosidic bond in doxorubicin splits and produces water insoluble aglycone (adriamycinone) and a water soluble, basic, reducing aminosugar (daunosamine).[19] However, the detection limits of this method exceed that obtained by a visible-light system method for detecting doxorubicin on skin and other surfaces developed by Van Raalte et al.[20] Van Raalte's method is based on detection of the fluorescence of doxorubicin HCl and is able to detect 20 ng on 80 mm^2 or 0.25 ng/mm^2 (25 ng/cm^2). The method using Whatman circle filters and solvent blend for wiping and desorbing doxorubicin HCl has a minimum concentration of detection of 2.0 ng/cm^2, and after converting units from ng/cm^2 to ng/mm^2, the minimum concentration of detection is 0.02 ng/mm^2.

Paclitaxel has very good lower limits for minimum concentration of detection on the different surfaces tested (S.D. <3.0 on vinyl flooring and stainless steel surfaces, and S.D. ≤ 8.7 on resin surface). What variation was observed may be due to the presence of a confounding agent such as another taxane (over 50 taxanes have been compiled from the studies of natural extracts for taxanes).[21] Such variations in paclitaxel results are more likely due to the low solubility of paclitaxel. Paclitaxel has limited solubility in aqueous solutions and can form white precipitate as well as degrade in the presence of light. It was very difficult to dissolve the paclitaxel in standards prepared for this study, and it is believed this low solubility is a factor in being able to readily recover paclitaxel from the surfaces tested. One existing method described in the literature for monitoring paclitaxel uses a blend of 200 µL of glacial acetic acid and methanol to make 1.0 L of acidified methanol reagent to dissolve the paclitaxel.[22] However, this blend would not be acceptable for use with the other agents of interest.

The Kimwipes were evaluated for all five agents on stainless steel, but it was found that what appears to be one of the additives used in the manufacture of the Kim-wipes elutriates at the same point in time on the HPLC chromatogram as ifosphamide. Therefore, Kimwipes were determined to not be acceptable as surface-monitoring media for the antineoplastics reviewed in this study.

One concern in using Whatman filters for sampling media is its durability, that is, many of the filters seem to decompose during use. Although that did occur with the Whatman grade #42 circle filters, it was to a minimal extent and did not interfere with analysis so long as appropriate syringe filters were used in removing the desorbate from the desorbing vials.

When detection limits for the Whatman method were compared to the other existing methods and adjusted for the difference in surface area, the minimum concentration of detection was much closer than expected to more sensitive methods. When results of the Whatman filter wipes for detection of cyclophosphamide were compared with the method used by Connor et al., detection appeared to be even more acceptable. That is, the 1.7 ng/cm^2 minimum concentration for detection for a 600 cm^2 resin (~0.65 ft^2) surface wiped by Whatman filter, when adjusted as if wiping 4900 cm^2 (~760 in^2 or ~5.3 ft^2) and the same relative quantity of agent recovered per given area, it would equal ~0.1 ng/cm^2.

Connors' method is more sensitive for the same area at a minimum limit of detection of ~0.01 ng/cm^2, but it involves an extraction step followed by analysis by GC in tandem with mass spectroscopy-mass spectroscopy (GC-MS-MS).[9] The advantages of the Whatman circle filter and HPLC surface monitoring method are in ease of use and ability to detect five agents simultaneously, and it requires less analytical time to conduct than use of the method involving a GC-MS-MS system. Further, although the GC-MS-MS analysis does have high specificity and sensitivity, it requires a derivatisation step such as use of trifluoroacetic anhydride for derivatisation of ifosphamide and cyclophosphamide.[23] It also requires relatively expensive and complex analytical instrumentation and is not as readily able to conduct simultaneous analyses for five different agents like the HPLC method.

Results of this study also confirmed the effectiveness of using a mixed solvent for both the wipe procedure and sample desorbing for detection of the five different agents of interest. Based on this information, it appears likely that this method can be used for detection of many of the other cytostatic drugs on similar surface types (e.g., methotrex-

tection of many of the other cytostatic drugs on similar surface types (e.g., methotrex-ate and ganciclovir).

Application of method. It should be mentioned that recent findings of surface contamination from monitoring in areas where these agents are not actually handled but may be handled in adjacent areas reinforce information that some biological safety cabinets (BSCs) do not effectively control some cytostatic drugs. Specifically, a class II, Type A BSC recirculates approximately 70% of cabinet air through high efficiency particulate air (HEPA) filters back into the cabinet; the rest is discharged through a HEPA filter into the preparation room. Schmidt reported in July 1999 that the cyclophosphamide particles appeared to sublimate off the HEPA filter and return to (e.g., recondense in) the work area.[24] This observation is somewhat supported by the findings of. Connor et al, where he states in his study of six cancer treatment centers in Canada and the United States, that "contamination was observed in areas adjacent to the work areas."[9] Thus, in conducting an evaluation of the pharmacy and administration areas of an oncology treatment area for potential exposure to the health care workers, monitoring should be conducted in adjacent areas as well as in the pharmacy and administration areas. This is especially important where cyclophosphamide and ifosphamide are used in a class II, Type A BSC.

Wipe sampling can be a very useful tool in determining those areas where antineoplastic agents are used that may either necessitate additional decontamination of surfaces or the use of PPE such as gloves and aprons used by individuals working in such areas. Wipe sampling technology can also be applied to evaluation of PPE to verify ef-

fectiveness of the equipment (e.g., wipe test and analysis of inside surfaces of gloves). Detection of antineoplastic agents on the inside of gloves would indicate glove failure and that the gloves should be replaced with a more protective type (e.g., thicker material) or be changed more frequently by the user.

Areas where antineoplastic agents are used generally require special cleaning and decontamination procedures.[2] Frequently, the cleaning requires use of either a detergent for polar compounds, a solvent for the non polar compounds, or the combination of both when polar and non polar compounds are both likely to be present in order to first remove the agent(s). This is usually followed by use of a decontamination step, which frequently involves use of sodium hypochlorite such as a 10% bleach solution (a dilution of bleach containing 5.25% sodium hypochlorite diluted 10:1 with water to ~0.5%). The final step is repeating the wipe sampling to verify the cleaning and decontamination steps were effective in removal of the potential hazard.

In the development and evaluation of this method, a review of the literature was conducted to identify what other methods have been developed and used for surface monitoring. Several methods were identified. The OSHA Technical Manual (Section II, Chapter 2,[6] extensively revised and featuring new additions, 1994) indicates classic wiping techniques and media for collection of wipes samples from surfaces. Mixed cellulose ester filter discs (AA filters) or smear tabs are most often recommended. However, squares of a gauze material are sometimes used because of their being more durable than filter media, especially when wiping rough surfaces. Either the mixed cellulose ester or the gauze material may be used dry or wet with an appropriate solvent (usually 50% solution of isopropyl alcohol in water).[2]

An example of a method found to have been successfully used is one by Sessink et al., which uses 20 x 21 cm tissues (Kleenex professional wipes, Kimberly Clark Corp., Koblenz, FRG) for wipe sampling.[8] The tissue is wet with a sodium hydroxide solution (0.03 M), and the spots and objects are swept clean. For the packings, boxes, chamber pots and urinals, two tissues and 5 mL of the sodium hydroxide solution were used. The floors, working trays of hoods, the tables, and the sink units were cleaned with four tissues and 10 mL of sodium hydroxide solution. For wipe samples taken from the floor, the tissues were not wetted but the solution was pipetted on the floor. The minimum concentration of detection for cyclophosphamide and 5-fluorouracil using this wipe sampling method was indicated to be (1) 0.02 ng/cm^2 cyclophosphamide and 0.1 ng/cm^2 5-fluorouracil on a 0.7 x 0.7 m area (27.6 x 27.6 inches or ~5.3 ft^2) of floor and tables (2) 0.01 ng/cm^2 cyclophosphamide and 0.04 ng/cm^2 5-fluorouracil on a 1.9 x 0.8 m area (74.8 x 31.5 inches or 16.36 ft^2) of working trays of two hoods, and (3) 0.06 ng/cm^2 cyclophosphamide and 0.3 ng/cm^2 5-fluorouracil on boxes and 9 cyclophosphamide drug vials and 20 5-fluorouracil ampules before and 76 packings after preparation of drugs.

The method used by Connor et al.,[10] as previously discussed, measures an area of 4900 cm^2, spreads a solution of 0.03 M sodium hydroxide over the area with a pipette, typically 20 mL, and uses one or two Scott 130 roll towels (Kimberly-Clark Corp., Northrop, UK) to wipe the measured surface.[9] The towel (or towels) is stored in a coded, 125-mL plastic screw-top container (Nalge Nunc). All samples were extracted and analyses were conducted by GC-MS-MS. Minimum concentrations of detection are similar for those indicated in the method used by Sessink et al.

CONCLUSIONS

This method for surface monitoring is recommended because it is relatively easy and cost-effective (requires less time and less costly equipment) to perform and has very good precision and accuracy for the water soluble alkylating agents ifosphamide and cyclophosphamide.[25] It can be a useful tool in identifying the presence of these agents on the types of surfaces indicated to determine the level of exposure risk and to evaluate the effectiveness of methods used to remove and clean these agents from such surfaces.

Methods utilizing sodium hydroxide solution for removal and GC-MS-MS (tandem Mass Spectroscopy) for analysis are slightly more sensitive and are able to attain lower limits of detection in ng/cm^2. However, this appears to be due in part to larger surface areas being wiped than 600 cm^2 used in this method. This method utilizing Whatman grade # 42 circle filters, is accurate and sensitive enough for evaluation of surface contamination on various types of surfaces typically found in both pharmacy and administration areas of oncology treatment facilities. It is also able to determine if areas handling such agents have been acceptably cleaned after use.

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A NEW MONITORING METHOD USING SOLID SORBENT MEDIA FOR EVALUATION OF AIRBORNE CYCLOPHOSPHAMIDE AND OTHER ANTINEOPLASTIC AGENTS

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ABSTRACT

Cyclophosphamide is a known human carcinogen. In July 1999, Schmidt[1] (Dulsburg, Germany) gave a report at a conference on cytotoxic drugs in Sweden, indicating he found cyclophosphamide was not effectively controlled by high efficiency particulate air (HEPA) filters. This then raised a concern that the existing air monitoring methods, which utilize polytetrafluoroethylene (a.k.a. PTFE or Teflon) or glass fiber filters for evaluation of antineoplastics such as cyclophosphamide in air, may also be ineffective for collection and quantification of such agents. After review of the information from Schmidt, it was decided that further evaluation of the existing filter method for monitoring antineoplastics in air was needed to confirm their effectiveness. It was determined that the filter method of monitoring was minimally effective for some antineoplastic agents, and that an alternate method of monitoring should be sought or developed. The method subsequently developed utilizes a solid sorbent tube, Anasorb 708 (a.k.a. Chromosorb 108, Ambersorb XAD-8), which is a methacrylic acid polymer. Evaluation of this sorbent tube for adsorption and desorption properties found it had a greater than 95% recovery for cyclophosphamide and greater than 90% for ifosfamide. Other agents evaluated included 5-flurouracil, doxorubicin, and paclitaxel. These additional three agents could also be detected and measured by use of the Anasorb 708 solid sorbent tube. However, paclitaxel can be effectively monitored by the filter method. Validation of the method was then conducted using spiked Anasorb 708 tubes as sources and Anasorb 708 as collection media. Air was pulled through the tubes via attachment to an air manifold system, with airflows ranging from 1.5 to ~4.0 L/minute for up to 24 hours. This evaluation did validate the Anasorb 708 tube as a very effective media for collection of air-

borne concentrations of cyclophosphamide from less than 1 μg up to approximately 2 mg (2000 μg)per tube, corresponding to a concentration range of approximately 0.7 $\mu g/m^3$ (0.0007 mg/m^3) to 0.7 mg/m^3 in a 5.76 m^3 volume of air. This method is able to provide accurate information on airborne concentrations of cyclophosphamide for purposes of conducting risk assessments or evaluation of risk management methods.

BACKGROUND

Cyclophosphamide, which is one of the most frequently used antineoplastic agents in clinical treatment facilities,[2,3] and a known human carcinogen (International Agency for Research on Cancer [IARC] class 1-A),[4,5] was determined to be a primary agent in need of further method development. Other agents were included in the study based on their potential health risks and frequency of use. These were ifosfamide, fluorouracil, doxorubicin, and paclitaxel.

Because of the physical properties of cyclophosphamide, it was previously thought that it could only exist in air as a particulate. Typical monitoring consisted of using either 0.5 μm glass fiber filters or 0.45 μm polytetrafluoroethylene (PTFE; Teflon) filters.[6-11] Samples were usually collected over periods of at least 40 hours and frequently collected for more than 80 hours. Results generally were very low or below limits of detection, even when monitoring in areas where high volumes of cyclophosphamide were being used.[11] Further, the National Institutes of Health recommended that work with hazardous drugs, which includes cyclophosphamide, be carried out in a biological safety cabinet (BSC), which is equipped with high efficiency particulate air (HEPA) filters to control the agents.[13]

In July 1999, Schmidt gave a report at a conference on Occupational Exposure to Cytotoxic Drugs in Sweden.[1] He indicated that he found cyclophosphamide and possibly other antineoplastic agents are not effectively controlled by HEPA filters. Such filters are used for control of aerosol contaminants in class II, type A BSCs.[12] These cabinets recirculate approximately 70% of cabinet air through HEPA filters into the cabinet, and the remainder is discharged through HEPA filters into the room. Schmidt speculated that the cyclophosphamide particles are probably captured by the HEPA filters as particles and than evaporate or sublimate off the filter, returning to the local air through the BSC exhaust streams.

This observation is somewhat supported by Connors et al. recent work on surface contamination.[13] They have found several areas in cancer centers that have surface contamination from antineoplastic agents. In addition, a 1999 article by Sessink and Bos stated that "uptake of cyclophosphamide was even found in pharmacy technicians involved in the preparation of cytostatic drugs other than cyclophosphamide."[8] This would indicate they were likely being exposed by either airborne concentrations of cyclophosphamide or from surfaces contaminated with cyclophosphamide. Based on the observations of Schmidt, exposure was likely occurring by both mechanisms.

Because of these findings, it was concluded that, if cyclophosphamide was not effectively collected and controlled by HEPA filters in BSCs, there may be a flaw in existing air monitoring methods, which utilize PTFE or glass fiber filters for collection. If it were correct that cyclophosphamide evaporated or sublimated off the filters, it was likely that the filter monitoring method is not effective for monitoring cyclophosphamide, and an alternate monitoring method would need to be considered.

An evaluation of the effectiveness of the filter monitoring methods currently in use was conducted. Results indicated the filter monitoring method to be minimally effective for collection of cyclophosphamide. Based on this information, it was decided that a method using a solid sorbent might be more effective for collection of the cyclophosphamide as a vapor. Thus, steps were taken to develop a monitoring method in which solid sorbents would be used as an effective sample collection media for cyclophosphamide, as well as for some other commonly used antineoplastic agents. Because of their potential toxicity and common use in cancer therapy, ifosfamide, 5-fluorouracil, doxorubicin hydrochloride, and paclitaxel[6-11,14,15] were included in development of a monitoring method acceptable for cyclophosphamide.

Air monitoring method considerations

Selection of air monitoring method. Methods available for consideration included use of an impinger to collect airborne contaminants in a solvent solution that could be directly injected into an analytical instrument, use of a filter collection method to collect airborne particles, or use of a solid sorbent to collect contaminants in the gas and/or vapor state. Use of impingers has a variety of drawbacks. For example, impingers can loose a portion of the collection solvent and are relatively easy to spill or the impinger may break. Therefore, it was decided to evaluate the other two types of air monitoring methods available, which are use of filters and use of solid sorbents for monitoring of airborne antineoplastics of interest.

Analytical method

Analytical equipment. Analytical methods reviewed included reversed-phase high performance liquid chromatography (HPLC), gas chromatography (GC), GC-mass spectroscopy (GC-MS) and GC-mass spectroscopy-mass spectroscopy or GC-tandem mass spectroscopy (GC-MS-MS).[6-11] The GC-MS-MS and the GC-MS have very high specificity and sensitivity, but they do have some restrictions. Complicated sample handling, often requiring derivatisation, may be required in order to avoid the decomposition of agents like cyclophosphamide on the column.[6,8] Although the GC method was determined to have the sensitivity needed to measure low microgram concentrations of an antineoplastic, it does not have the versatility desired to simultaneously detect multiple agents having different chemical characteristics because of the derivatisation step. Thus, it was decided that the reversed-phase HPLC would likely be the optimum method for simultaneous analysis of the five agents of interest.

A Bio-Rad reversed-phase HPLC equipped with a model 1790 programmable ultraviolet-visible spectrophotmeter (UV/VIS) monitor was used. The system utilized a 500 μL injection loop and a Waters 150 x 4.6 mm Symmetry C18 column with 5.0 μm particles.[17]

Mobile phase. A combination isocratic and gradient method was found acceptable for detection of all five agents with a single analysis. The first 20 minutes used a mobile phase of 22.75% acetonitrile (ACN) in Milli-Q water, with the water buffered by potassium phosphate to a pH 6.0 at a flow rate of 1.2 mL/minute. This concentration of ACN in water and associated flow rate was found to provide the separation of all but one

agent peak in 20 minutes. This isocratic phase was followed by a 25-minute gradient phase to a 70% ACN and buffered water to elute the last agent, paclitaxel. The gradient was then brought back to 22.75% over an additional 5 minutes, followed by a 10-minute period to completely return to the normal baseline. Gas formation from the ACN was minimized by first degassing any solutions containing ACN.

Preparation of standards. The selectivity of the method was verified by the analysis of blank and spiked samples. Quantification (peak height) was carried out by reference to calibration curves constructed from the analysis of freshly prepared reference samples. These reference samples varied from containing an individual agent at a specific concentration to containing all five agents at select concentrations. The standards used for controls were prepared from the products indicated in Table 1.

Table 1.
Information on Agents Used to Make Standards

Brand Name	Composition	Lot No.	Manufacturer
Cytoxan (for injection)	Cyclophosphamide (20 mg/mL)	Lot 9G22823	Meade-Johnson (Bristol Myers Sq.)
Ifex (for injection)	Ifosphamide (50 mg/mL)	Lot KCS99	Meade-Johnson (Bristol Myers Sq.)
Doxorubicin HCl	Doxorubicin HCl (2 mg/mL)	Lot 123200A Lot 93592	Novaplus Bedford Labs
Adricil (for injection)	Fluorouracil (50 mg/mL)	Lot FFA221	Pharmacia & Upjohn
Paclitaxel (99.99%)	Paclitaxel	Not Available	Sigma

Each of these five agents was first prepared by dilution in a blend of one part methanol and one part Milli-Q water (some products had to be reconstituted first with distilled water before initial preparation as standards). Any subsequent dilutions to prepare standard of a desired concentration were obtained by blending with Milli-Q water.

Determination of UV wavelength for detector. Each of these agents (Table 2) was scanned by spectrophotometry (Beckman DU-600) to determine their individual optimum UV wavelength range in nanometers (nm) for UV detection. It was learned that these agents were generally most detectable in the range between 190 and 200 nm. Based on this information, the HPLC UV detector was set at 195 nm for detection.

Table 2.
Optimum Ultraviolet (UV) Detection Range for the Agents of Interest

Agent	UV Wavelength (nm)
Fluorouracil	195 to 210
Ifosphamide	190 to 195
Cyclophosphamide	190 to 195
Doxorubicin	190 to 195, and 230 to 235
Paclitaxel	190 to 210

Selection of desorbing solution. Typically, the solution used for desorbing sorbents from either air or surface sampling media has been 50% methanol:50% water buffered to pH 6.0 by blending monobasic and dibasic potassium phosphate.[6-11] Because of the variation in physical characteristics of the agents of interest in this study, especially

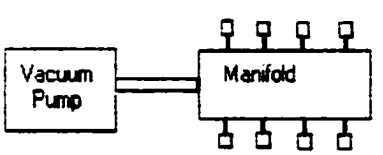
considering the range of polar sites, various concentrations of ACN and methanol in water were tested for desorbing capabilities. It was determined that the most effective desorbent was a blend of 10% ACN, 25% methanol, and 65% Milli-Q water. This blend assured optimum desorption of cyclophosphamide and ifosfamide and acceptable desorption of 5-fluorouracil, doxorubicin, and paclitaxel (Pac).

Evaluation of overall effectiveness of monitoring medias

One important step in determining the effectiveness of a collection media for the monitoring of a contaminant in air is to evaluate the ability of the sorbent to retain the contaminant(s). Specifically, the ability of the collection material to retain the captured contaminant as air continues to be pulled through the sorbent after the contaminant is no longer present in the air. Because of the potential for cyclophosphamide to sublimate off filter material, this was an important test for this study.

Air manifold system. To conduct controlled testing of the various air monitoring methods, an air manifold was constructed using a 15-inch length of 4-inch PVC pipe, with a cap glued on one end and a screw-on cover at the opposite end. This pipe was then equipped with 8 needle valves to control airflow at each air inlet port (See Figure 1).

Figure 1. Air manifold system connected to a vacuum pump with inlets to the manifold equipped with needle valves for flow control of air through each sample test train.



The manifold was connected to a vacuum pump, which was able to pull up to 30 L/min total air volume through the eight ports on the manifold. A Dry-Cal calibration unit was used to measure airflow rates at each of the needle valve ports on the manifold. Once the airflows were known for each inlet, the sampling train to be evaluated was attached to the port. After the attachment of the sample train, the airflows were remeasured at the inlet of the train. All manifold outlets used were measured after any adjustment to any outlet before initiation of the testing. A final measurement was made immediately prior to removal of the sample trains from the manifold ports. The initial and final measurements were averaged and then multiplied by the time in minutes to obtain the total volume of air moved through the sample train.

Filter method evaluation

PVC filter evaluation. The first material tested as a possible monitoring media was a 37 mm, 5.0 μm PVC filter. The PVC filter was tested both with and without a support pad, and the support pad was tested without a PVC filter. In each case, the filter and/or support pad was spiked with 2.0 mg cyclophosphamide. An Anasorb 708 tube was connected downstream of the filter cassette, and air was pulled through the sample train, typically for 16-17 hours.

The results for both the filter analyses and Anasorb 708 analyses are found in Table 3. Results indicate approximately 500 μg of the 2000 μg spike was lost off the PVC filter and support pad, the filter without a support pad, and support pad without a filter after approximately $2m^3$ of air was pulled through each. Note that a control filter was spiked with 2000 μg, stored at ambient temperatures but with no air movement through

Table 3.
PVC Filter Tests: 2000 μg Spike of Cyclophosphamide

Sample No.	Sample Description	Air Flow L/Min	Air Vol. (m^3)	Cyclophosphamide Mass Recovered (μg)
1	PVC Filter & Support Pad	2.16	2.19	1530
2	PVC Filter, No Support Pad	2.02	2.07	1510
3	PVC Support Pad, No Filter	2.36	2.41	1490
Anasorb 708 Tubes After Filters				
4	After filter - 1	2.16	2.19	<0.2
5	After filter - 2	2.02	2.07	<0.2
6	After filter - 3	2.36	2.41	<0.2
Control		None	None	1980

PVC filters: 37 mm diameter, 5.0 μm pore size, polyvinyl chloride filter circle.

it, and desorbed. Results yielded 1980 μg or nearly 100% recovery. Because the cyclophosphamide lost from the PVC filters was not detected in the respective Anasorb 708 tubes, it was suspected that the lost cyclophosphamide either sublimated onto the sidewalls of connection tubing or sidewalls of filter cassette or both. This was confirmed as indicated in Tables 14 through 17.

PTFE (Teflon) filter evaluation. Tested next for potential use as monitoring media was a 37 mm, 0.45 μm PTFE filter in cassettes. Also evaluated were the cellulose support pads for the PTFE filters. These filters and support pads were each dosed with 2 mg of cyclophosphamide and evaluated. Approximately 5 m^3 of air (3350 cc/minute for 1490 minutes) was pulled through the filter and/or support pads (see Table 4). Anasorb 708 tubes were placed after the PTFE filters to determine if the cyclophosphamide was being lost to the air exhausting from the filter cassette.

Results from this test found that the PTFE filter and the PTFE support pad without a filter each lost nearly 1700 μg from an initial loading of 2000 μg (2.0 mg) or greater

than an 83% loss after 5 m^3 of air pulled through the PTFE filter cassette assembly. It was also noted that only a very small fraction of the cyclophosphamide loss was detected in the Anasorb 708 tubes downstream of the spiked filter assembly.

Table 4.
Results from Evaluation of PTFE Filters and Support Pads to Determine Stability of 2000 µg Cyclophosphamide Spiked onto the Filter After Air Pulled Through.

Sample No.	Sample Description	Cyclophosphamide Mass Recovered (µg)
PTFE (Teflon) Media Tests		
1	PTFE Filter & Support Pad – 2.0 mg CP	335.4
2	PTFE Support Pad only – 2.0 mg CP	324.9
3	Blank TEFLON Support Pad, no air	<0.2
4	Field Blank Anasorb 708	<0.2
Analyses of Anasorb 708 After Support Pad Spiked With 2.0 mg CP		
1	Anasorb 708, First tube after Support Pad	4.9
2	Anasorb 708, First tube after Support Pad	1.8
3	Anasorb 708, Second tube after Support Pad	<0.2
Note: Field blank kept in admin office for 24 hours with ends open		

PTFE Filer: A 37 mm dia., 0.45 µm pore size, PTFE (Teflon) filter circle.

Results in table 5 indicate air pulled through the PTFE filter alone reduced the concentration from 2.0 mg cyclophosphamide to approximately 200 µg. This indicates a loss of cyclophosphamide off the PTFE filters only of approximately 90% due to air being pulled through the filter (see Table 5). This is a greater loss than that off the filter and support pad or support pad only.

After analyses of the spiked PTFE filters and/or support pads and the Anasorb 708 collection media, it appeared that a significant amount of cyclophosphamide mass had been removed from the filter. There was only a minimal amount of cyclophosphamide mass collected on the Anasorb 708 media downstream of the spiked filters. For

Table 5.
Results from PTFE Filters Only to Determine Stability of 2000 μg Cyclophosphamide Spiked onto the Filter After Air Pulled Through.

Sample No.	Sample Description	Cyclophosphamide Mass Recovered (μg)
PTFE & Anasorb 708 Media Tests		
1	PTFE Filter Source #1 – 2.0 mg CP	209
2	Anasorb 708, first tube after filter source #1	2
3	PTFE Filter Source #2 – 2.0 mg CP	192
4	Anasorb 708, first tube after Filter source #2	1

PTFE Filer: A 37 mm dia., 0.45 μm pore size, PTFE (Teflon) filter circle.

purposes of mass balance, that is, to identify where the cyclophosphamide had moved to, the filter cassettes were rinsed, and the rinse was analyzed. Results from analyses of the rinse found a significant amount of the original cyclophosphamide mass was on the interior walls of the cassettes (average of 175 ug cyclophosphamide). Since filters were found to be inefficient collection media, solid sorbents were selected for evaluation.

Evaluation of solid sorbents

The first step in evaluation of solid sorbents was to select an acceptable method for determining the optimum sorbent. The method used in this study for evaluation of the sorbents was generally based on procedures developed in the 1970s by the United States National Institute for Occupational Safety and Health (NIOSH).[18,19] In this method, air spiked with contaminants of interest is drawn through a sample tube containing a sorbent material. After the sorbent potentially traps the contaminants, it is later desorbed and analyzed to determine acceptability, both in recovery and sensitivity. Development of a method using a solid sorbent for air monitoring has a number of advantages. Lightweight pumps and sample tubes are relatively cheap and user-acceptable. Increasing the flow

rate or time of sampling can increase sensitivity. Many different chemicals can be sampled using a single sorbent, and a time-weighted average is possible over relatively short periods (e.g., 8 hours).[20] The next step was selecting sorbents to test.

Selection of sorbent. The initial selection of sorbents to be evaluated was focused on monitoring of cyclophosphamide. This was because cyclophosphamide is a known human carcinogen, is widely used and in relatively large quantities, and was therefore being evaluated as a marker of antineoplastic agent contamination. However, because of the interest in also being able to detect and measure other contaminants of interest, it is important to identify a sorbent that has the capability for adsorption and subsequent desorption of other agents in addition to cyclophosphamide.

In 1996, Stanetzek et al.[21] evaluated several adsorbents for specific breakthrough and retention volumes and for their respective adsorption enthalpies for various organic compounds. Adsorption enthalpy is a measure of the strength of the adsorptive interactions with the adsorbent surface.

One of the organic chemicals evaluated in the study by Stanetzek et al., which has a chemical structure somewhat similar to cyclophosphamide, was cyclohexylamine. Cyclohexylamine ($C_6H_{13}N$) has a molecular weight of 99.2, and is a polar chemical.[22] Cyclophosphamide ($C_7H_{15}C_{l2}N_2O_2P(H_2O)$ has a molecular weight of 279.1 and is also polar.[21] Although the molecular weights are different, the structures are similar in that each has a single ring and both have nitrogen molecules attached to the ring.

Reviewing the characteristics related to specific retention and breakthrough volumes for a variety of solute and adsorbent combinations, three solid sorbents appeared to

be candidates for screening. These are Anasorb 708 (Amberlite XAD-8), Porapak R and activated carbon (see Table 6).

Table 6.
Properties of Candidate Sorbents for Cyclophosphamide Monitoring

Sorbent Name	Amberlite XAD-8 (Anasorb 708)	Porapak R	Activated Carbon
Specific surface area (m^2/g)	140	450-600	1000
Average pore diameter (Å)	250	76	Not Obtainable
Monomer Composition	Methacrylic acid polymer	N-vinyl pyrrolidine polymer	Carbon (Coconut charcoal)
Adsorption Enthalpy (ΔH) (re; Cyclohexylamine)	70.5-kJ/mol	55-kJ/mol	No Values
Retention (V_g) & Breakthrough volumes (V_D), & Specific breakthrough volume (re; Cyclohexylamine)	560 V_g(V_D) 310 (L/g)	330 V_g 330 (L/g)	No Values Decomposition Possible
MESH SIZE	Not Available	50/80	Not Available

Ref: Stanetzek et al., 1996[21]

Note: V_g = Retention Volume (Retention time of solute molecule (t_R or min/gram) multiplied by the mobile phase flow rate (F or L/min), or L/g).

V_D = Breakthrough Volume (Breakthrough time of solute molecule, which is when it is first detectable at the column outlet (t_D or min/gram) multiplied by the mobile phase flow rate (F or L/min), or L/g).

L/g = Specific Breakthrough Volume.

Cyclophosphamide being a polar material requires a sorbent having both favorable adsorption and desorption properties. Since the interest also is in simultaneous monitoring of other antineoplastic agents, some of which are non polar, it was also necessary to try to identify a sample media that had characteristics acceptable for effective sampling and desorption of non polar agents as well as polar.

Evaluation of solid sorbents. Evaluation of the sorbents was a multiphase activity. The first phase was to determine the ability for the antineoplastics of interest, especially cyclophosphamide, to be desorbed from the sorbent. This phase would likely determine which, if any, of the first three solid sorbents selected for evaluation would be acceptable. After this phase, the next was to evaluate the collection ability of the sorbent found that is able to be acceptably desorbed. The final phase was to conduct a field evaluation of the method in a cancer center or infusion therapy area to determine if other agents used in such areas may cause interference with the method.

Sorbents selected for testing

As indicated earlier, three sorbents were selected for testing. All three sorbents were obtained from SKC, Inc., Eighty Four, PA. Specific information on each sorbent is indicated in Table 7. Initially, tests were conducted on each sorbent at various concentrations of antineoplastic agents to obtain a rough indication of the ability for adsorption and desorption for each agent. Results from this spiking and desorbing of the sorbents at varying concentrations are shown in Tables 8-10.

Table 7.

Information on Each of the Three Solid Sorbents in Sampling Tubes Tested

Sorbent Name	Exp. Date	Tube Size (mm)	Sorbent Quantity (mg)	Catalog Number
Amberlite XAD-8 (Anasorb 708 or Chromasorb 108)	4/04	6 x 70	100 (Lot 1124)	226-30-08
Porapak R	4/04	6 x 70	35/70 (Lot 896)	226-59-04
Anasorb CSC (coconut charcoal)	8/04	8 x 110	350/350/350 (Lot 2000)	226-09-02

All sorbents purchased from SKC South, Appomattox, VA. (SKC, Inc. Headquarters, Eighty Four, PA.).

Evaluation of sorbents for desorption acceptability. Procedures for evaluating the sorbents began with spiking each sorbent with a specific concentration of cyclophosphamide. The spiked sorbent tubes were sealed (capped) and stored at ambient temperatures for at least 12 hours. The tubes were then cut open, and the sorbent was put into a desorbing vial (a clear 10 mL glass Microvial with a screw-on cap and septum, manufactured by Kimble Glass, Inc. Art. No. 60710-10). Typically, 2 mL of desorbing solvent was injected into each vial for desorption of the contaminants. The solvent blend determined to have optimum desorption capabilities, based on trials of various blends, was a mixture of 10% ACN, 25% methanol, and 65% Milli-Q water. After the solvent was added, the vial(s) were place on an orbital shaker (Lab-line orbital shaker) and shaken for 30 minutes at 150 rpm. After shaking, the desorbate was removed via a syringe equipped with a syringe filter (Whatman 13 mm Syringe Filter, 0.2 um PVDF Filter Media , Sterile and Non-pyrogenic, Catalog No. 6791-1302) and placed into a 15-mL disposable tube

with a screw-on cap (Corning disposable centrifuge tubes w/flat cap, non-pyrogenic polypropylene, catalog no. 430790). After desorbing, analysis of the desorbate was conducted by the analytical method developed earlier.

Activated carbon. Activated carbon was evaluated first by injection of 10-μg of 5-fluorouracil, ifosfamide, cyclophosphamide, and doxorubicin HCl and 5 μg of paclitaxel. After desorption in 2.0 mL of desorbent, if able to be effectively desorbed, the expected results would be 5.0 μg/mL of ifosfamide, cyclophosphamide, and doxorubicin HCl and 2.5 μg/mL 5-fluorouracil and paclitaxel if 100% desorption. When results at this concentration were found to be poor, the sorbent was tested at 100 μg for Ifosfamide, 40 μg for cyclophosphamide and doxorubicin HCl, and 4 μg of paclitaxel, but this time desorption was with 3 mL of desorbing solution (Table 8). This would provide an expected ifosfamide concentration of 33.3 μg/mL, 13.3 μg/mL for cyclophosphamide and doxorubicin HCl and 1.33 μg/mL paclitaxel. Results for this concentration were also poor. It was noted that the 5-fluorouracil could not be detected at either concentration due to its peak being hidden in the solvent peak at the beginning of the chromatogram.

Table 8.
Desorption Results for Activated Carbon After Spiking with Agents

Sample No.	Sample Description	Mass Recovered (μg) FU	IF	CP	DX	Pac
1	Blank Activated Carbon tube	<0.2	<0.2	<0.2	<0.5	<0.2
2	5.0 μg IF,CP,DX; 2.5 μg FU, Pac	<0.2	<0.2	<0.2	<0.5	3.5
3	33.3 μg IF; 13.3 CP,DX; 1.33 μg Pac	<0.2	<0.2	<0.2	<0.5	2.1

Note: Fluorouracil (FU) results are hidden in solvent peak
FU = 5-Fluorouracil, IF = Ifosfamide, CP = Cyclophosphamide
DX = Doxorubicin HCl, Pac = Paclitaxel

These results indicated an inability for activated carbon to be effectively desorbed for ifosfamide, cyclophosphamide, and doxorubicin HCl and possibly 5-fluorouracil. Therefore, activated carbon was found to be unacceptable as a solid sorbent for monitoring the antineoplastic agents of interest in this study.

Porapak R. Results from screening tests on Porapak R tubes for use in monitoring antineoplastic agents are shown in Table 9. Like the activated carbon tubes, these tubes were tested blank (no spike) and then with different concentrations of the antineoplastic agents of interest to determine desorption efficiency. Porapak R was only able to detect ifosfamide and cyclophosphamide at concentrations at or above 25 μg/mL.

Table 9
Desorption Results for Porapak R Tubes

Sample No.	Sample Description		Mass Recovered (μg) FU	IF	CP	DX	Pac
1	Blank Porapak R tube	0.05	<0.2	<0.2	<0.2	<0.5	<0.2
2	5.0 μg/mL IF,CP,DX; 2.5 FU, Pac	0.05	<0.2	<0.2	<0.2	<0.5	<0.2
3	5.0 μg/mL IF,CP,DX; 2.5 FU, Pac	0.05	<0.2	<0.2	<0.2	<0.5	<0.2
4	25 μg/mL IF, FU; 10 CP,DX; 1 Pac	0.05	<0.2	30.9	15.3	2.15	1.5

Note: Fluorouracil peak hidden in solvent peak
FU = 5-Fluorouracil, IF = Ifosfamide, CP = Cyclophosphamide
DX = Doxorubicin HCl, Pac = Paclitaxel

Anasorb 708. Results from the screening tests on Anasorb 708 (Chromasorb 108, XAD-8) were very good. Results indicated detection from the initial testing at spike concentrations of 1.0 ug/mL for ifosfamide, cyclophosphamide and paclitaxel. Results from the screening tests are shown in Table 10.

Table 10.
Results for Anasorb 708 (Chromasorb 108, XAD-8)

Sample No.	Sample Description	Mass Recovered (µg) FU	IF	CP	DX	Pac
1	Blank Anasorb 708 tube	<0.2.	<0.2	<0.2	<0.5	<0.2
2	1.0 ug IF,CP,DX; 0.5 µg FU, Pac	<0.2	1.5	1.3	0.2	0.7
3	1.25 µg IF,CP,DX, FU; 0.626 µg Pac	<0.2	1.1	0.9	0.1	2.1

Note: Fluorouracil results were hidden in solvent peak
FU = 5-Fluorouracil, IF = Ifosfamide, CP = Cyclophosphamide
DX = Doxorubicin HCl, Pac = Paclitaxel

Further evaluation of Anasorb 708. Based on these screening test results for Anasorb 708, it was decided to conduct spiking and analyses on a set of five tubes of this sorbent for a more accurate evaluation (see Table 11).

Table 11.
Recovery Evaluation Results for Five Anasorb 708 (Chromasorb 108) Tubes

Sample No.	Mass Recovered (µg) IF	CP	DX	Pac
1	22.4	9.1	5.0	1.0
2	21.4	7.2	12.0	1.1
3	24.4	7.8	13.0	0.8
4	24.6	7.5	10.0	0.8
5	23.9	10.1	9.0	0.7
Average Recovered (µg):	23.3	8.3	9.8	0.9
Average Recovery (%):	93.3	83.3	98.0	87.0
S.D. (ug):	1.4	1.2	3.1	0.2
Coeff. of Variation (C.V.(%)):	5.9	14.4	31.8	19.2
Amount Spiked (µg):	25.0	10.0	10.0	1.0

IF = Ifosfamide, CP = Cyclophosphamide
DX = Doxorubicin HCl, Pac = Paclitaxel

Results from the five tube analyses showed that the average recoveries of cyclophosphamide, ifosfamide, doxorubicin HCl and paclitaxel from Anasorb 107 were quite

acceptable, ranging from 83.3% for cyclo-phosphamide to 98% for doxorubicin HCl. However, precision and accuracy was very poor for doxorubicin HCl, and marginal for ifosfamide and paclitaxel. 5-Fluorouracil was also added to each spiked tube at a concentration of 25 ug, but it was not detected. It is believed the solvent peak on the chromatogram hid the 5-fluorouracil peak. Anasorb 708 was determined to be the sorbent of choice because of the collection and desorption capabilities demonstrated for it.

Results from the five samples above provide information on the accuracy and precision in desorption of ifosfamide and cyclophosphamide. The average recovery for ifosfamide at a concentration of 25 μg was 22.86 μg or 91.4% average recovery. Bias was 6.7%, and precision based on the coefficient of variation (C.V.) was 5.9%. Accuracy, based on bias of 8.6% (bias is equal to100% minus average recovery of 9.4%) plus twice precision (2 x C.V.), was 20.3%. The average recovery for cyclophosphamide at a concentration of 10 μg was 8.3 μg or 83.3% average recovery.

Precision was estimated to be 14.4%, with a bias of 16.7%. Accuracy was estimated at 45.5%. Average recovery for doxorubicin was 98%, but the precision was 31.7%, and estimated accuracy was 65.5%.

Average recovery for paclitaxel was acceptable at 87%, but, again, there was a fairly wide range of results. Precision for paclitaxel was estimated at 19.18%, and estimated accuracy was 51.36%. Fluorouracil could not be evaluated because its peak could not be separated from the solvent peak on the chromatogram at the concentration tested (25 μg).

Although the solid sorbent media were able to detect paclitaxel from the injected concentration, because of its physical characteristics it was determined that paclitaxel

would not sublimate or evaporate. And since it would remain a particulate, continued use of the filter method would be a more accurate method for collection of paclitaxel in air. Based on this conclusion, paclitaxel was not included in subsequent tests for this method.

Validation of Anasorb 708 for monitoring antineoplastics

Six Anasorb 708 tubes were spiked with 200 μg of cyclophosphamide each. Air was then pulled through each of them, into and through another Anasorb 708 tube for downstream collection of any agent that might be released from the spiked tubes (see Figure 2). After pulling air through the spiked tubes into and through the collection tubes, all tubes were desorbed with 2.0 mL of desorbent each. Results for the spiked tubes after air was pulled through them are shown in Table 12.

Table 12.
Results from Evaluation of Anasorb 708

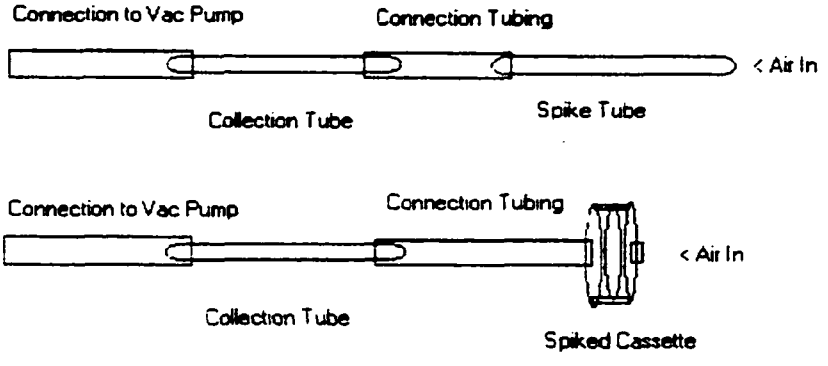
Sample No.	Sample Description (Flow Rate)	Air Vol.(m^3)	Cyclophosphamide Mass Recovered (μg)
1	Spike #1 (1704 cc/minute)	2.13	161.5
2	Spike #2 (2541 cc/minute)	3.17	274.7
3	Spike #3 (2929 cc/minute)	3.65	263.9
4	Spike #4 (3186 cc/minute)	3.97	151.2
5	Spike #5 (1830 cc/minute)	2.28	138.3
6	Spike #6 (2061 cc/minute)	2.57	150.0
Average Mass Recovered (μg):			189.9
Standard Deviation (μg):			62.0
Coefficient of Variation (C.V.(%)):			32.6
Spiked Quantity (μg):			200.0

Note: Period for pulling air through spiked tubes was 1247 minutes for each tube.

Cyclophosphamide was not detected in any of the downstream sample tubes but was detected only in the spiked tubes. The average recovery results from this testing of

Anasorb 708 for effectiveness in collecting cyclophosphamide was approximately 190 μg from a 200 μg spike or 95%. However, precision (based on C.V.) was 32.6%, and the estimated accuracy was 75.3. This lower precision and accuracy was attributed, at least in part, to the likelihood that the capacity of the 100 mg Anasorb 708 sorbent tube was greater than 200 μg, Thus, the mass of 200 μg of cyclophosphamide injection did not exceed the capacity of the spiked tube to allow for excess cyclophosphamide to be released from the tube and collected in downstream tubes. Based on this observation, it was decided to spike the Anasorb 708 tubes, while the manifold system was operating, with a larger mass of each agent of interest (see Figure 2).

Figure 2. Typical arrangement of sample test train, with spiked media (filter cassettes or solid sorbents) ahead of solid sorbent collection media and ahead of vacuum pump.



Anasorb 708 collection tubes were placed behind the tube to be spiked on the sample train. A syringe was used to inject the antineoplastic mass into the first set of tubes as air was being pulled through them. A total mass of 5.0 mg of 5-fluorouracil, ifosfamide, and cyclophosphamide and 1.0 mg of doxorubicin HCl was added slowly to each Anasorb 708 tube to be spiked during the evaluation period. The average flow rate

(cc/minute), period of the test (minutes), and the total volume for each series of tubes and connecting tubing, otherwise referred to as the test sample train is shown in Table 13.

Table 13.
Flow Rates, Test Period and Total Volume of Air for Each of Six Sample Sets

Sample No.	Air Flow Rate (L/min)	Sample Time (min)	Sample Vol.(m^3)
1	1.80	1330	2.21
2	1.66	1330	2.39
3	1.91	1435	2.75
4	1.88	1435	2.70
5	2.76	1435	3.96
6	2.37	1435	3.40

Note: flow rates were chosen that would be comparable to those likely to be used in actual monitoring situations.

The collection and retention abilities of the combination of spiked source tubes, connecting tubing, and both collection tubes are shown for each agent in Tables 14 through 17 and Figures 3 through 6.

Table 14.
Analytical Recovery Results for Fluorouracil from Test Sample Train, 5.0 mg Spike onto Anasorb 708 Tubes

Sample No.	Air Vol. (m^3)	Mass Recovered (mg)			
		Initial	Connectings	Collectors	Total
1	2.21	2.0	1.8	1.6	5.4
2	2.39	2.0	1.3	1.7	5.0
3	2.75	2.0	0.9	2.3	5.2
4	2.69	2.0	1.0	2.3	5.3
5	3.96	2.0	1.2	1.5	4.7
6	3.40	2.0	0.8	2.2	5.0
Avg Recovered (mg):		2.0	1.2	1.9	5.1
Avg Recovery (%):		40	23	39	102
S.D. (mg):		<0.1	0.4	0.4	0.3
C.V. (%)		0.9	31.2	20.3	4.9

Bias of 10.0%, precision (based on C.V. of 4.9%), and estimated accuracy of 19.8%.

Table 15.
Analytical Recovery Results for Ifosfamide from Test Sample Train, 5.0 mg Spike onto Anasorb 708 Tubes

Sample No.	Air Vol. (m^3)	Mass Recovered (mg)			
		Initial	Connectings	Collectors	Total
1	2.21	2.2	1.0	1.6	4.8
2	2.39	2.2	1.0	0.5	3.7
3	2.75	2.2	0.9	2.2	5.3
4	2.69	2.2	1.3	2.2	5.7
5	3.96	2.2	0.9	0.8	3.9
6	3.40	2.2	1.1	1.9	5.2
Avg Recovery (mg):		2.2	1.0	1.5	4.8
Avg Recovery (%)		44	20	30	95
S.D. (mg):		< 0.1	0.1	0.7	0.8
C.V. (%):		0.5	13.6	47.0	16.9

Bias of 4.8%, precision (based on C.V. of 16.9%), and estimated accuracy of 38.7%.

Table 16.
Analytical Recovery Results for Cyclophosphamide from Sample Train, 5.0 mg Spike onto Anasorb 708 Tubes

Sample No.	Air Vol. (m^3)	Mass Recovered (mg) Initial	Connectings	Collectors	Total
1	2.21	2.2	0.4	0.9	3.4
2	2.39	2.2	0.7	0.8	3.7
3	2.75	2.1	0.8	1.9	4.9
4	2.69	2.2	0.8	1.7	4.6
5	3.96	2.2	1.1	0.8	4.1
6	3.40	2.2	0.8	1.4	4.4
Avg Recovered (mg):		2.2	0.8	1.3	4.2
Avg Recovered (%):		44	15	25	84
S.D. (mg):		< 0.1	0.2	0.5	0.6
C.V. (%):		1.1	31.7	39.2	13.2

Bias of16.3%, precision (based on C.V. of 13.2%), and estimated accuracy of 42.7%.

Table 17.
Analytical Recovery Results for Doxorubicin from Test Train, 2.0 mg Spike onto Anasorb 708 Tubes

Sample No.	Air Vol. (m^3)	Mass Recovered (mg) Initial	Connectings	Collectors	Total
1	2.21	0.8	0.2	<0.1	1.0
2	2.39	0.7	0.4	<0.1	1.1
3	2.75	0.4	<0.1	1.2	1.6
4	2.69	0.5	0.4	<0.1	0.9
5	3.96	1.0	0.3	<0.1	1.2
6	3.40	0.8	<0.1	0.5	1.3
Avg Recovery (mg):		0.7	0.2	0.3	1.2
Avg Recovery (%)		34.0	10.5	14.5	60.0
S.D. (mg):		0.2	0.2	0.5	.3
C.V. (%):		29.6	87.0	170.8	21.3

Bias of 40.5%, precision (based on C.V. of 21.3%), and estimated accuracy of 83.1%.

Figure 3. Recovery of 5-Fluorouracil from different sample train components.

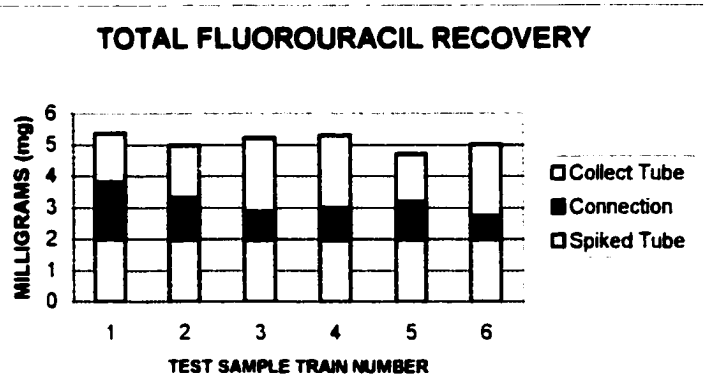


Figure 4. Recovery of ifosfamide from different sample train components.

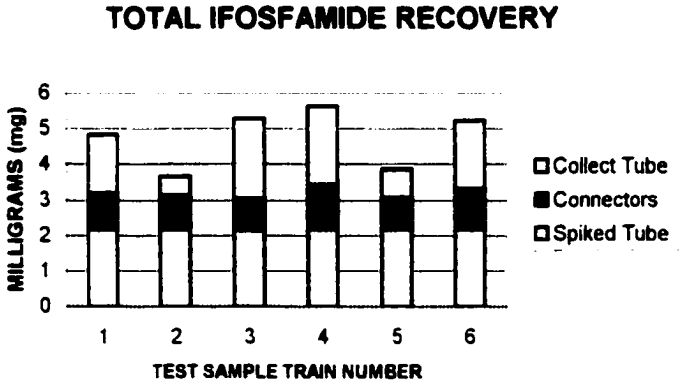


Figure 5. Recovery of cyclophosphamide from different sample train components.

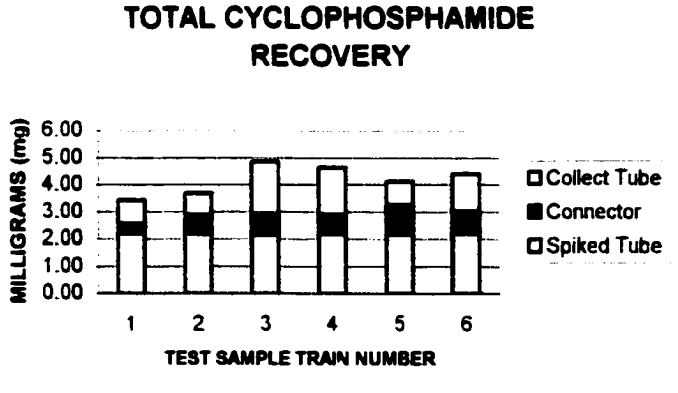
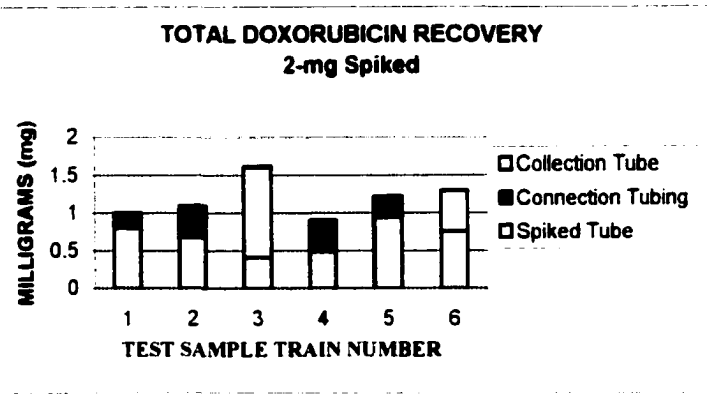


Figure 6. Recovery of doxorubicin from different sample train components



DISCUSSION AND CONCLUSIONS

Historically, based on the physical characteristics for agents such as cyclophosphamide, it was believed that these agents remained in particulate form; thus, the use of filters for monitoring would be most effective. However, the information obtained during the development of this method confirms the observations by Schmidt.[12] That is, cyclophosphamide may be captured by air filters but then sublimate or evaporate off the filter. In such a case, even if diffusion of the cyclophosphamide particles occurs at the surface of the filter, once it sublimates the filter surface area is miniscule compared to that of the surface of the solid sorbent. Consequently, collection of the now gaseous molecules by the filter is inefficient. This explains why monitoring results for cyclophosphamide from methods utilizing filters were generally very low or below detection limits, even when the monitoring periods were for relatively long periods of time (such as several days).

In addition to the filter method not being acceptable for air monitoring for cyclophosphamide, and possibly some of the other antineoplastics, such as ifosfamide and fluorouracil, this information also supports the observation that the HEPA filter is not an

acceptable control for BSCs that return filtered air to the work area. Because of this, cyclophosphamide exposures to health care professionals are likely to have been occurring, even when results from long-term air monitoring by filter method of the work environment indicated no or very low exposures to be occurring.

Further, results from the monitoring in the infusion therapy clinic's oncology pharmacy preparation hood (BSC) was likely to have been compromised due to the attachment of the filter cassette with filter ahead of the Anasorb 708 tube. The cyclophosphamide would likely go through the filter, but some could be attracted to the interior sidewalls of the filter cassette. Thus, a reduced concentration would reach the Anasorb 708 tube. It is important that there be no cassette or tubing ahead of the inlet to the Anasorb 708 tube when monitoring for cyclophosphamide, ifosfamide, or fluorouracil.

This study found that the method utilizing Anasorb 708 solid sorbent for air monitoring is accurate to measure ifosfamide and cyclophosphamide concentrations from less than 1.0 ug/mL to >1.0 mg/mL. This provides a monitoring range of <0.35 $\mu g/m^3$ to approximately 350 $\mu g/m^3$ when sampling is conducted for 24 hours at 2 L/minute. Thus, the lower limit of detection for cyclophosphamide provides adequate sensitivity to detect concentrations of cyclophosphamide in air well below the recommended exposure limit of 0.001 mg/m^3 (1.0 ug/m^3). Further, it is also acceptable for monitoring fluorouracil and ifosfamide.

Results from analysis of the spiked Anasorb 708 tubes indicated the maximum capacity of cyclophosphamide to be a consistent 2.17 mg, with a standard deviation of 0.02 mg and a coefficient of variation of 1.08%. This was observed with air volumes pulled through the tubes in the range from 2.2 to 3.9 m^3. This indicates very good preci-

sion and accuracy over a relative wide range of air volume. Similar results were seen for fluorouracil and ifosfamide. Average tube capacity for fluorouracil was 1.99 mg, with a S.D. of 0.02 mg and C.V. of 0.88%. Average tube capacity for ifosfamide was 2.18 mg, with a S.D. of 0.01 and C.V. of 0.54%.

This method may be acceptable for monitoring doxorubicin, but further evaluation is needed due to the relatively rapid decomposition of doxorubicin, especially when it is in contact with other chemical agents.

Based on desorption efficiency and capacity tests, this method has the sensitivity and precision to conduct assessments for airborne concentrations of not only cyclophosphamide but also fluorouracil and ifosfamide wherever they may be used. Further, high accuracy can be expected for cyclophosphamide, fluorouracil, and ifosfamide based on the consistency in the mass of each recovered (the capacity) from the spiked tubes in lieu of a wide range of total air volume pulled through each tube (from ~2.2 m^3 to ~4.0 m^3). This method can therefore be used to provide credible exposure information for use in epidemiological studies, risk assessment studies, risk management purposes, and establishment of compliance standards based on dependable information. Relative to risk management, availability of accurate monitoring data can also provide important information on the effectiveness of various engineering and administrative exposure controls available where cyclophosphamide is or may be handled.

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DISCUSSION, CONCLUSIONS, AND RECOMMENDATIONS

Discussion

As indicated in the section Goals and Objectives, there were two major goals: (1) enhance or develop an acceptable analytical method for simultaneous analysis of all five agents and (2) develop acceptable sample collection methods for both surfaces and air. This involved the need to meet 14 objectives.

The first three objectives related to the first goal, development of the analytical method. All three were successfully completed: (1) optimum operating conditions for the HPLC were identified, (2) recovery and stability tests were conducted, and (3) the analytical method was conducted on samples collected in an oncology pharmacy and in cancer treatment areas where additional agents would be used and be potential confounders in the analysis.

The next five objectives related to surface monitoring method development.

(1) An acceptable material, the Whatman #42 ashless circle filter was identified as meeting that objective.

(2) After many trials, a suitable reagent was identified as acceptable for sample wipe purposes.

(3) It was determined the same reagent used for sample wipes could be used for sample desorption purposes.

(4) Successful testing was completed on examples of the three surfaces typically found in hospital pharmacy or cancer treatment facilities.

(5) Testing was conducted in an oncology pharmacy area and a cancer treatment clinic.

It was observed during these tests that the doxorubicin HCl appeared to deteriorate rapidly when in contact with the vinyl floor tile and the resin surfaces. This may have been due to the contact with chemical components used in the construction of these materials.

Four objectives were associated with air sampling method development. The results of work to meet these four objectives are listed below:

(1) Evaluation of the filter method to collect some antineoplastics such as fluorouracil, ifosfamide, cyclophosphamide and doxorubicin were found to significantly underindicate their presence in air.

(2) Alternate methods for air monitoring were reviewed, but solid sorbent was decided to be the most likely method to be acceptable. The Anasorb 708, which is methyl acrylate polymer sorbent, was determined to be the most effective.

(3) The Anasorb tube was evaluated for both collection and desorption efficiency and found to have very acceptable ranges of detection for all five agents.

(4) Testing was conducted in an oncology pharmacy area and a cancer treatment clinic, with detection levels below 1.0 $\mu g/m^3$ for cyclophosphamide, which is the exposure limit for antineoplastic agents recommended by the National Institute for Occupational Safety and Health (NIOSH).

It was noted both in the lab and during the comparison testing of monitoring methods in the oncology pharmacy area and cancer treatment clinic that the Teflon filter method appeared to be as accurate as the solid sorbent for monitoring paclitaxel. That is

possibly due to the paclitaxel being relatively insoluble and not volatilizing or sublimating as the other agents appeared to do off or through the filter.

The last two objectives were to test the methods in an oncology pharmacy area and a cancer treatment clinic, and to publish the results of this research.

Surface monitoring was conducted in both an oncology pharmacy area and in a cancer treatment clinic, but the oncology pharmacy had more significant air and surface contamination detected than in the clinic. This is believed due to the use of a class II BSC in the oncology pharmacy area. Results of the studies at a hospital oncology pharmacy are listed in Table 1.

Oncology pharmacy surface monitoring results. Surface monitoring was conducted using Whatman #42 filter paper circles (55 mm dia.) to wipe a 600 cm^2 area. Results are shown in Table 1.

Table 1.
Monitoring Results on Various Surfaces in a Hospital Oncology Pharmacy Area

Sample No	Sample Location	Cyclophosphamide	
		Total ug	ug/100cm^2
1	Inside, base (flooring) of Chemo BSC (hood)	4.14	0.69
2	Center area of Injection Counter	3.86	0.64
3	Back corner of Injection Table	3.60	0.60
4	Back corner of Chemo table	Trace	Trace
5	Floor, front right side of Chemo BSC	46.35	7.70
6	Wall, right side of Chemo BSC, 5 ft above Floor	2.76	0.46
7	Front of storage containers (Random), Near BSC	Trace	Trace

N.D. = non detected.
National Institute of Occupational Safety and Health (NIOSH) Recommended exposure limit for surfaces is Non-Detectable

Oncology pharmacy air monitoring results

Air monitoring was conducted in three locations using SKC Double Take sampling pumps. Each sampler contained two pump devices able to be individually calibrated. One pump was attached to a filter cassette containing Teflon filter, 0.45 um pore size, and a cellulose support pad. The other pump in the sampler was equipped with solid sorbent. This dual monitoring using the filter method with the solid sorbent method was conducted to determine the differences in the two under actual test conditions. The respective results of this monitoring are indicated in Table 2.

Table 2.
Air Monitoring Results Comparing Filter Monitoring Method to Solid Sorbent (Anasorb 708) Monitoring Method in a Hospital Oncology Pharmacy Area

Sample No.	Location	Cyclophhosphamide (ug)	Vol. Air (m^3)	Concentration (ug/m^3)
Solid Sorbent				
1.	Exhaust from Chemo BSC (hood)	3.59	2.31	1.55
2.	Front of second shelf, Injection Counter	N.D.	2.23	N.D.
3.	Inside Chemo BSC (Back, Left of Hood)	N.D.	2.23	N.D.
TEFLON Filter and Support Pad				
1.	Exhaust from Chemo BSC (hood)	N.D.	2.34	N.D.
2.	Front of second shelf, Injection Counter	N.D.	2.35	N.D.
3.	Inside Chemo BSC (Back, Left of Hood)	N.D.	2.25	N.D.

N.D. = non detected.
NIOSH Recommended exposure limit is 0.001 mg/m^3; for surface it is non detectable.

It is important to note that cyclophosphamide was indicated as being detected on the sidewalls of cassettes (used as filter housing when sampling with Teflon filters and support pads) when evaluating methods with spiked cyclophosphamide sources. Therefore, the cassettes used in the above sampling were rinsed with the desorbing solvent and

analyzed. Traces of what appears to be CP were detected in trace quantities, although cyclophosphamide was not actually detected on the Teflon filters.

It is also important to note that several other chemical agents were used in the oncology pharmacy area at the time of this air and surface monitoring evaluation, and several unidentified peaks were observed on the chromatogram. The peaks identified as being cyclophosphamide in the analytical chromatogram are based on the time the peak eluted on the sample analysis versus the time cyclophosphamide eluted from analysis of a cyclophosphamide standard. It is possible that the peak identified as cyclophosphamide could be another agent. The recommended method for verification is use of a gas chromatograph or HPLC connected to a mass spectroscopy unit to verify the composition of the peak at this point of elution.

Conclusions

The analytical method when used in conjunction with the surface and air sample collection methods described in the manuscripts above was very effective in determining the concentration of contaminants. These methods are relatively easy to use when compared to several other methods, which required complex manipulation (e.g., sample preparation for GC-MS analysis). The blend of solvents (10% ACN, 25% methanol, and 65% water) was a significant improvement in solutions used for removal, as well as for the desorption of a mix of agents having different chemical properties. These methods also have the ability to detect concentration of the agents studied at low enough concentrations on surfaces and/or in air to make the information very useful in the evaluation of the various types of exposure controls that are available for use.

A number of risk assessments have also been conducted on oncology nurses and related health care workers involved in handling antineoplastic agents. However, most have indicated that the exposures in their work areas were low and frequently non detected. This research finds that the more likely situation was that the agents were present but not detected because of the monitoring methods used. The use of filters for collection of cyclophosphamide in air was found to yield inaccurate results, and the surface monitoring methods where only 100 cm^2 was wiped were also very limited in the value of the information generated. Additionally, the surface monitoring methods appeared to have also been limited by the type of solution used to wipe surfaces. Therefore, the availability and use of these methods is expected to provide more accurate information on health care workers' potential for exposure, and this information can provide government agencies with accurate information to aid in development of an acceptable exposure limits for these agents.

It should be mentioned that good personal hygiene and cleanup of facilities and equipment was conducted in accordance with UAB requirements for working with hazardous agents.[165] That is, all materials used in this testing, including test materials, dilutions of test materials, containers in contact with antineoplastic agents, bench pads, latex gloves, Tyvek lab gowns and paper products used for cleanup, etc. were placed in plastic bags and returned to the UAB hazardous waste incineration container.

Recommendations

It was determined during evaluation of the data generated during conducting this research that it would have been beneficial to have used an internal standard to verify the

agent peaks on the chromatogram, especially when several samples need to be analyzed and more than one day is needed. A slight shift in peaks was noticed when the same samples were analyzed on different days.

Additional research should be considered on the types of filtration material that could be used to replace or augment the HEPA filters on BSCs that exhaust a portion of filtered air back to the work area. Use of activated carbon, which was found in the sorbent evaluations to strongly absorb the antineoplastics like cyclophosphamide and ifosfamide, or methyl acrylate polymer as used in the sampling media may be beneficial sorbents for increasing filter efficiency of the agents of concern.

The air monitoring method that was identified as being most acceptable was based on the demonstration that cyclophosphamide and most likely some of the other agents of interest sublimate. This sublimation of the agent from a solid to gas form allows for their effective capture of the agent as a gas in the solid sorbent. Further investigation should be considered to evaluate monitoring methods that would collect the agents such as cyclophosphamide in both their solid and gas states. Such a method may be a single monitoring tube containing both an integral glass fiber filter and a methyl acrylate polymer and both monitoring medias desorbed together as a single monitoring device. This would also avoid loss of solid agent being removed by electrostatic factors that appear to arise in a filter cassette, which negates the use of such a device ahead of the solid sorbent.

Additional research should be considered to determine what other chemotherapy agents may be monitored using the methods described above. It was observed when using the monitoring methods in the actual cancer treatment facilities that several other chromatogram peaks appeared, indicating the presence of other agents. The determina-

tion of other agents able to be detected by the above methods and the respective sensitivity to detect each would expand their use for both exposure evaluation and risk assessment.

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APPENDIX

LIST OF MORE FREQUENTLY USED AND POTENTIALLY HAZARDOUS ANTINEOPLASTICS USED AT A HOSPITAL ONCOLOGY PHARMACY AND AT AN INFSION THERAPY CLINIC PHARMACY

LIST OF CHEMOTHERAPY TREATMENT (ANTINEOPLASTIC) DRUGS BY GROUPING
(Agents of potential exposure concern to health care professionals)

GROUP	GENERIC NAME (Trade Names)	USE AT/DOSE Hospital = H Clinic = C	PURPOSE (Treatment for)	POTENTIAL EFFECTS
Antimetabolite				
Pyrimidine Analog		C		
	5-Flourouracil (Adrucil, Efudex, Fluoroplex)	500 mg/10mL	Colorectal, rectum, breast, stomach, and pancreas cancer	Bone pain (due to increased blood cell production, high tox. and potential birth defects
Folic Acid Analog				
	Methotrexate (Folex, Mexate)	H & C 9 mg to 14 g	Antimetabolitic agent; affects growth of cancer cells	Decreased blood count, mouth ulcers, nausea & vomiting
Alkylating Agent				
Nitrogen Mustard				
	Cyclophosphamide (Cytoxan, Neosar)	H 100 mg to 6 g	Lymphomaa, multiple myeloma leukemias, lupus, rheum-arthr mycosis fungoides	Nausea, decreased blood Count, cystitis, cardiac and lung tox. red spots on skin, cough/hoarse
Nitrogen Mustard				
	Ifosfamide (Ifex)	H 1 g to 6 g	Germ cell-Testicular cancer	Severe myelosuppression, hematologic and neuro toxicity
Ethyleneimine				
	Thiotepa (Thioplex)	H 300 to 400 mg	Breast, ovary, urinary bladder cancers	Decreased blood count, mouth ulcers, nausea & vomiting, hair loss, and cardiac toxicity

GROUP	GENERIC NAME (Trade Names)	USE AT/DOSE Hospital = H Clinic = C	PURPOSE (Treatment for)	POTENTIAL EFFECTS
Alkylating Agent (Continued)				
Triazine				
	Dacarbazine (DTIC-Dome)	C 200 mg	Misc. cancers	Low blood counts, nausea and vomiting, Hair Loss, Liver difunction, anorexia
Miscellaneous Agents				
Platinum-Coordinated complexes				
	Carboplatin (Paraplatin)	C 50 to 450 mg	Metastatic testicular tumors, or ovarian tumors, bladder Ca	Nausea, decreased blood count, liver and lung toxicity
	Cisplatin (Platinol)	H & C 35 to 2000 mg	Metastatic testicular tumors, or ovarian tumors, bladder Ca	Nausea, decreased blood count, liver and lung toxicity
Natural Products				
Antibiotic				
	Doxorubicin HCl (Adriamycin RDF)	H & C 50 to 150 mg	Acute lymphoblastic leukemia, Wilms' tumor, neuroblatoma, breast and ovarian carcinoma	Irreversible myocardial toxicity, low blood counts, nausea and vomiting, hair loss
Epipodophylotoxin				
	Etoposide (Vepesid)	H & C	Misc. cancers (e.g., small cell lung cancer)	Myelo suppression, hair loss, gastrointestinal and mouth ulcers
Diterpenoid Compound				
	Paclitaxel (Taxol)	C 30 to 100 mg	AIDS-related Kaposi's sarcoma, ovarian, breast, head, cancers	Bone marrow depression, cardiac toxicity

GROUP	GENERIC NAME (Trade Names)	USE AT/DOSE Hospital = H Clinic = C	PURPOSE (Treatment for)	POTENTIAL EFFECTS
Natural Products (Continued)				
Vinca Alkaloid				
	Vinblastine Sulfate (Velban, Velsar, Lilly)	H & C 8 to 10 mg	Testicular carcinoma, Kaposi's sarcoma, histiocytic lymphoma, Hodgkin's disease, mycosis fungoides	fever, chills, back or side pain blood in urine, unusual bruising red spots on skin, cough/hoarse
	Vincristine (Oncovin)	H & C 1 to 2 mg	Hodgkin's disease, non-Hodkins malignant lymphomas, neuro blastoma, and Wilms' tumor	Central nervous system, hair loss nausea & vomiting, mouth ulcers
	Vinorelbine (Navelbine)	C 10 to 50 mg	Misc. cancers	Central nervous system, hair loss nausea & vomiting, mouth ulcers
Anti-herpes Virus				
	Ganciclovir (Cytovene)	H 50 to 500 mg	Antiviral, block viral reproductn (Used to treat cytomegalovirus in AIDS patients)	Retinal detachment, decrease in kidney function, bone marrow depression, brain & nervous syst (Seizures, Coma)
	A B V D Chemo (Combo of 4 drugs: Doxorubicir, Bleomycin, Vinblastine, Dacarbazine	H & C Varies (low mg)	Various cancers	Potential to cause birth defects possibility for secondary cancers

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I certify that I have read this document and examined the student regarding its content. In my opinion, this dissertation conforms to acceptable standards of scholarly presentation and is adequate in scope and quality, and the attainments of this student are such that he may be recommended for the degree of Doctor of Philosophy.

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