

**TOWARDS THE REDUCTION OF OCCUPATIONAL EXPOSURE TO
CYTOTOXIC DRUGS**

by

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Abstract

Background: One of the most powerful and widely used techniques in cancer treatment is the use of cytotoxic drugs in chemotherapy. These drugs are inherently hazardous with many of them causing carcinogenic, mutagenic or teratogenic health outcomes. Occupational exposure to cytotoxic drugs is of great concern due to their lack of selectivity between healthy and unhealthy cells. Widespread cytotoxic drug contamination has been reported in North America, Europe and Australia. Current cleaning protocols for hazardous antineoplastic drugs include the use of disinfectants and oxidizing agents, such as household bleach.

Aim: The thesis project focused on two objectives: 1) hypothesize and confirm potential hazardous by-products arising from cleaning cyclophosphamide, a widely used cytotoxic drug, with household bleach, a commonly used cleaning agent; 2) develop an effective and safe cleaning agent for cytotoxic drugs in order to prevent and eliminate exposure to these drugs.

Methods: The gas chromatograph mass spectrum (GC/MS) was used to analyze the decomposition of cyclophosphamide by household bleach (5.25% hypochlorite). The reaction was conducted in a test-tube and the by-products extracted and derivatized prior to analysis.

Multiple cleaning agent compositions were tested on 10x10cm stainless steel plates spiked with the two model cytotoxic drugs, cyclophosphamide and methotrexate. A wipe-sampling procedure was used to determine amount of contamination present on surface post cleaning with different cleaning agent compositions. Analysis of wipes was conducted on a liquid chromatography tandem mass spectrum (LC/MS/MS).

Results: Decomposition of cyclophosphamide by household bleach was determined to produce nor-nitrogen mustard. Production of nor-nitrogen mustard was determined to be dependant to reaction time and volume of household bleach used.

A safe and effective cleaning agent for cytotoxic drugs, which acts by dissolving the contamination and not by decomposition, was developed. The cleaning agent was determined to remove >98% (methotrexate) and >95% (cyclophosphamide) of contamination by using a one-wipe procedure. A

systematic cleaning protocol is recommended by using a two-wipe procedure followed by the use of isopropanol.

Conclusion: Decomposition of cytotoxic drugs on surface can lead to unwanted and hazardous exposure. Cytotoxic drug contamination can be safely removed from surfaces without decomposition.

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Co-authorship statement

The research, experimental, analysis and written documents were conducted by me, and in consultation with Dr. Winnie Chu.

A version of chapter two, *Potentially carcinogenic by-products arise from cleaning cyclophosphamide with household bleach*, will be submitted for publication, with authors Barzan C. and Chu W.

A version of chapter three, *Develop a cytotoxic cleaning agent*, will be submitted for publication and patent application, with authors Barzan C. and Chu W.

Chapter 1. Introduction

According to the US National Institute for Occupational Safety and Health (NIOSH), more than 50% of American workers in facilities that have chemotherapy agents could potentially be in regular contact with chemotherapy drugs [1]. The most commonly used drugs in chemotherapy are cytotoxic drugs (also referred to as antineoplastic agents). Historically, the incident rate for cancer has been constantly on the rise. The latest estimates represent an increase of incidence rates since 2009 for both deaths attributed to cancer as well as new occurrences of the disease. It is expected that there will be 173,800 new cases of cancer and 76,200 cancer deaths in 2010 [2]. The implications of this data, in combination with Canada's ageing population will result in more frequent use of chemotherapy. This form of treatment is the most effective approach to dealing with cancer. The increased use of these agents will increase the risk of exposure to the healthcare workers and the general population.

Antineoplastic agents

A cytotoxic or cytostatic drug is described as any agent that prevents cell division or causes a destructive action to a cell as well as inhibits or prevents the function of a cell, [3,4]. Cytotoxic drugs are mainly used in cancer treatment; however, they have been used in treating other types of disease, such as multiple sclerosis [3,5-7]. Most of the drugs are not tumor cell specific and many of them have carcinogenic, mutagenic and teratogenic effects [3,5,6,8,9]. Side effects caused by each cytotoxic drug is related to the mechanism of action in the human body and are classified in five major categories: alkylating agents, antibiotics, antimetabolites, free radical generators, and mitotic inhibitors [6,10-11]. For example, one of the most commonly used cytotoxic drugs, cyclophosphamide (CP), is an alkylating agent. In the body, CP is metabolized by cytochrome P450 enzymes to become an active alkylating agent. This agent reacts with guanine (one of the base units in the DNA strand) and hence stops the reproduction of DNA. Please see Figure 1.1 for a simplified reaction mechanism for the interaction [12]. Methotrexate, on the other hand, is an antibiotic which interferes with the reproduction of DNA strands. Cytotoxic drugs can cause acute effects, which may include eye irritation, nausea, vomiting and diarrhea [6,10]. These drugs can also cause chronic effects, the most significant one being cancer.

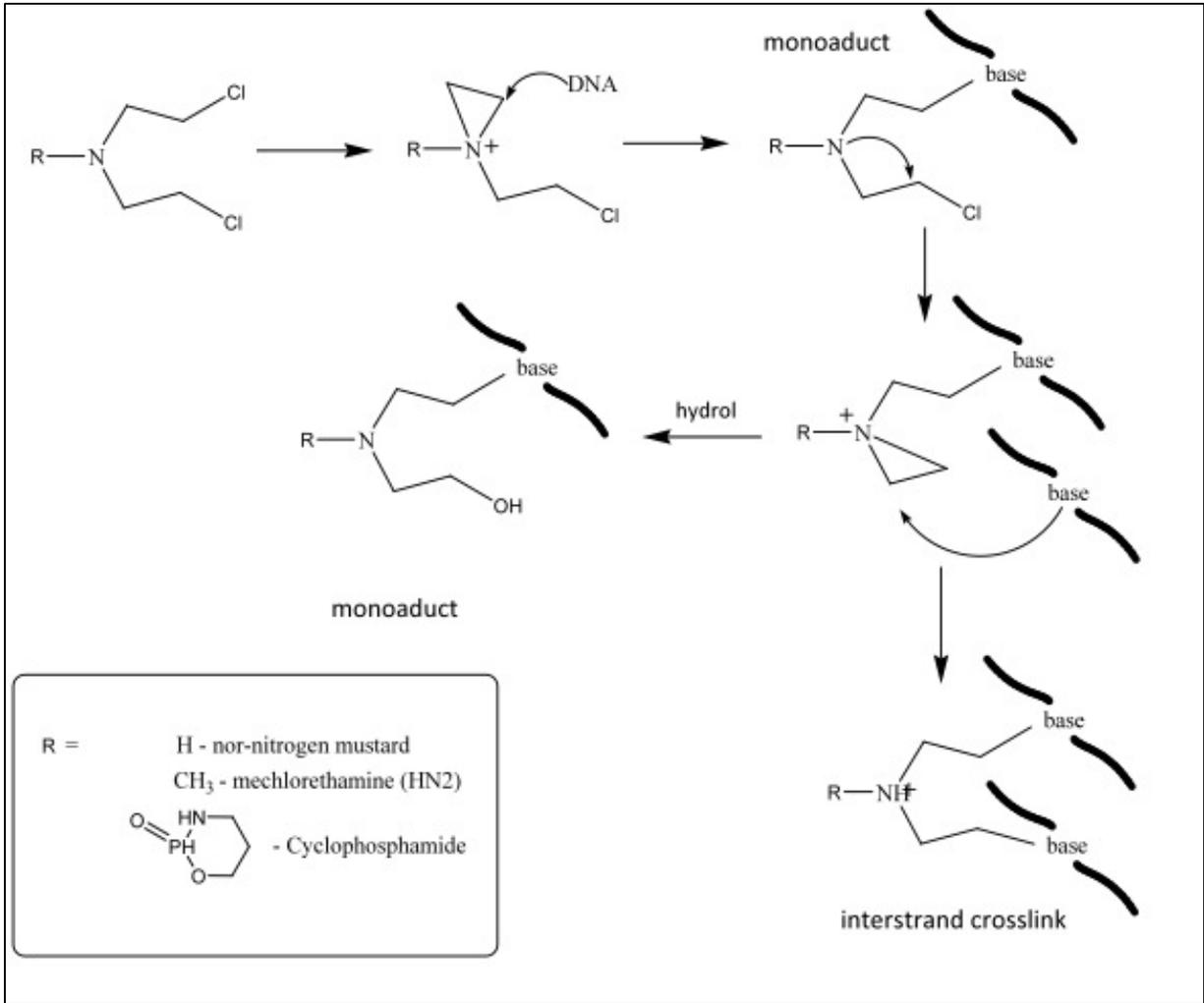


Figure 1.1 Simplified reaction mechanism for the reaction of nitrogen mustards with DNA [12]

Occupational exposure

The danger from occupational exposure to antineoplastic agents has been recognized since the 1970s [13]; however, the first safe handling guidelines for cytostatic drugs were not issued until 1981 by the Canadian Society of Hospital Pharmacists [14]. The effectiveness of cytotoxic drugs for treating cancer outweighs the risk to cancer patients from adverse side effects. But obviously the risks associated with these drugs cannot be overlooked in the case of exposure to healthcare workers or healthy members of the general public. According to the NIOSH, accumulation of adverse effects from low exposure to cytotoxic drugs is possible [5]. Furthermore, any level of exposure is deemed dangerous by safety regulators, as no acceptable level of exposure has been determined to be safe [3,5,7,15-17]. Numerous cytotoxic drugs are considered by the International Agency on Cancer Research as known carcinogens (Class 1), probable carcinogens (Class 2A) or possible carcinogens (Class 2B) [1,6,9,18-19].

Exposure to cytotoxic drugs has been shown to cause mutagenicity in workers [19-22]. Published literature has reported an increase in chromosomal aberrations as well as sister chromatid exchanges resulting from exposure [23-25]. These effects are known to be cumulative, and thus the total effect is based on past exposure. In occupational settings where chronic low-level exposure exists, the effects can increase and accumulate over time [26]. Furthermore, DNA damage has been reported as a result of handling cytotoxic drugs without proper protection [27-28].

Numerous occupational sectors have been identified as having a higher potential for exposure, ranging from the manufacturing process to the delivery, distribution of these drugs and disposal. The main routes of exposure are inhalation and skin absorption. The risk of exposure increases with increased frequency of use, lack of proper protection or improper use of protection. As well, the expanded use of these agents in human care, as well as in veterinary care, has contributed to occupational exposure risk factors [29].

Available literature from North America, Europe and Australia report cytotoxic contamination on a wide range of surfaces including: hospital floors (pharmacy, oncology and non-oncology), storage shelves, door handles and telephones [1,13,30-33]. Furthermore, contamination has been reported in locations where antineoplastic drugs are not handled or administered, such as reception areas. This increases the risk of exposure to anyone working in, or visiting, hospitals, veterinary care units or manufacturing plants [29].

As with any other occupational hazard, the first priority is substitution; thus removing the hazard in the first place and preventing the exposure. However, antineoplastic agents that are less harmful to workers without reducing the treatment effectiveness have not been discovered; which makes substitution not possible at this time [6]. Engineering controls have been developed and current WorkSafeBC regulations state that a Class II Type B biological safety cabinet must be used for cytotoxic drug preparation [4]. However, contamination with antineoplastic agents has been reported on numerous surfaces even with the use of these cabinets [1,29-30]. Ideally, occupational exposure to contaminated surfaces can be greatly reduced when proper cleaning protocols are applied.

Current cleaning protocols

The NIOSH recommends decontamination of hazardous products by a deactivation agent, but fails to define the requirements of an acceptable cleaning agent [5]. Household bleach is recommended on the NIOSH website, but with the disclaimer that it is not effective for all types of agents. The US Occupational Safety and Health Administration (OSHA) recommend the usage of alkaline detergent and 70% alcohol as part of the decontamination procedure [3]. Consistent standards for cleaning protocols or cleaning agents currently do not exist [3,5,32]. To date, only one commercially available cleaning agent, Surface Safe® with a two-step wipe cleaning procedure, claims to be capable specifically in the removal antineoplastic drugs [34]. The first wipe is impregnated with sodium hypochlorite 2%, which must be allowed to set for at least 30 seconds. The second wipe contains sodium thiosulphate, which is to be used to remove any hazardous by-products created by the hypochlorite. However, Hansel et al. reported sodium hypochlorite to require one hour in order to completely degrade cyclophosphamide. Thus, allowing only 30 seconds for cleaning will not provide nearly enough time. Furthermore, hypochlorite has been shown to be ineffective against compounds that cannot be oxidized.

In British Columbia, current cleaning procedures in hospitals include the use of household bleach (an oxidizing agent) or detergents (Chlorohexidine, Phenokil II® and Cavicide®) in combination with isopropanol [33]. Using bleach to remove surface contamination of cytotoxic drugs is the most commonly practiced cleaning protocol worldwide [34-43]. However, the cleaning efficiency of any of the above agents has not been determined. Therefore, the main objectives of this study were to test the cleaning efficiency of bleach and to investigate potential by-products, and to create an effective cleaning agent as part of a safe cleaning protocol suitable for real-world settings. Within the limited scope of the study, cleaning effectiveness was focused on the most commonly used cytotoxic drug:

cyclophosphamide. However, the developed cleaning agent was also tested against a structurally and functionally very different drug: methotrexate. Methotrexate is also a very commonly used chemotherapy drug.

Project rationale

Project I – Potentially carcinogenic by-products arise from cleaning cyclophosphamide with household bleach

Cyclophosphamide (Figure 1.2) is a known human carcinogen, classified by the international Agency for Research on Cancer (IARC) in 1981. With its wide spread use, cyclophosphamide (CP) is an ideal candidate to act as a model drug for occupational exposure determination

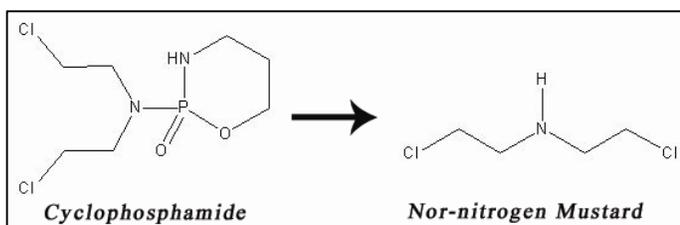


Figure 1.2 Proposed decomposition of cyclophosphamide

and assessment. Hypochlorite (HOCl) is the main ingredient in both the only

antineoplastic cleaning agent currently available on the market (Surface Safe®) and the internationally accepted cleaning agent (bleach) [34]. We hypothesize that the decomposition of cyclophosphamide by the oxidizing agent OCl⁻ may lead to the cleavage of the nitrogen-phosphorus bond, which creates nor-nitrogen Mustard (Figure 1.2).

Therefore, this project aimed to prove the reaction hypothesis by confirming the presence of nor-nitrogen mustard. This confirmation was performed by developing a sensitive instrument method to analyze both cyclophosphamide and nor-nitrogen mustard simultaneously in one solution. The determination of hazardous by-products from the initial reaction hypothesis demonstrates that the prediction process could be a powerful tool in designing drug cleaning agents. With this in mind, more detailed potential reaction mechanisms are also hypothesized to predict other by-products. Decomposition and deactivation of a toxic compound must not be recommended without first understanding the potential by-products.

Project II - Develop a cytotoxic cleaning agent

To remove cytotoxic drug contamination and hence reduce exposure, an effective cleaning agent which can be applied in real-world settings must be developed. Therefore, the development of a cleaning

agent that is safe and efficient in cleaning antineoplastic contamination was another main object of this study. Again, keeping the scope of the project in mind, testing the effectiveness of cleaning protocols was carried out on the most commonly used cytotoxic drug, cyclophosphamide. Methotrexate, another widely used drug, was also tested to determine the potential applicability of a successful cleaning agent to other cytotoxic drugs.

Detailed compound information

Cyclophosphamide

Cyclophosphamide (common trade names include: Cytoxan[®], Procytox[®], Neosar[®]) is a widely used alkylating agent and a derivative of nor-nitrogen mustard (Figure 1.3). The agent's primary use is to treat various types of cancer, including breast cancer, lung cancer, multiple myeloma, ovarian cancer, and various types of leukemia [5,45]. Furthermore, CP can also be used as an immunosuppressant to treat disorders that may include nephritic syndrome, Wegener's granulomatosis, and severe rheumatoid arthritis [4]. The primary mechanism of action consists

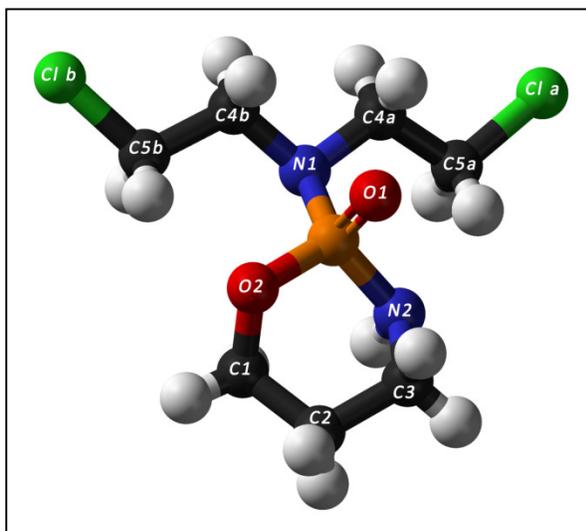


Figure 1.3 The 3-D model of cyclophosphamide with labelled coloured atoms (Carbon (black):C1 to C5; nitrogen (blue): N1; chlorine (green): Cl_a and Cl_b; oxygen (red): O1 and O2; hydrogen (Grey) and phosphorus (orange))

of activation to phosphoramidate mustard (the major alkylating metabolite from the reaction between cyclophosphamide and cytochrome P450 enzyme) in the body. This mustard

metabolite binds to DNA strands to form mono- and cross-linked DNA adducts (Figure 1.4). Please see Figure 1.4 for the activation of cyclophosphamide and its reaction with DNA. The agent inhibits protein synthesis through cross-linking between strands of DNA. Furthermore, phosphoramidate mustard undergoes cleavage to form nor-nitrogen mustard [46].

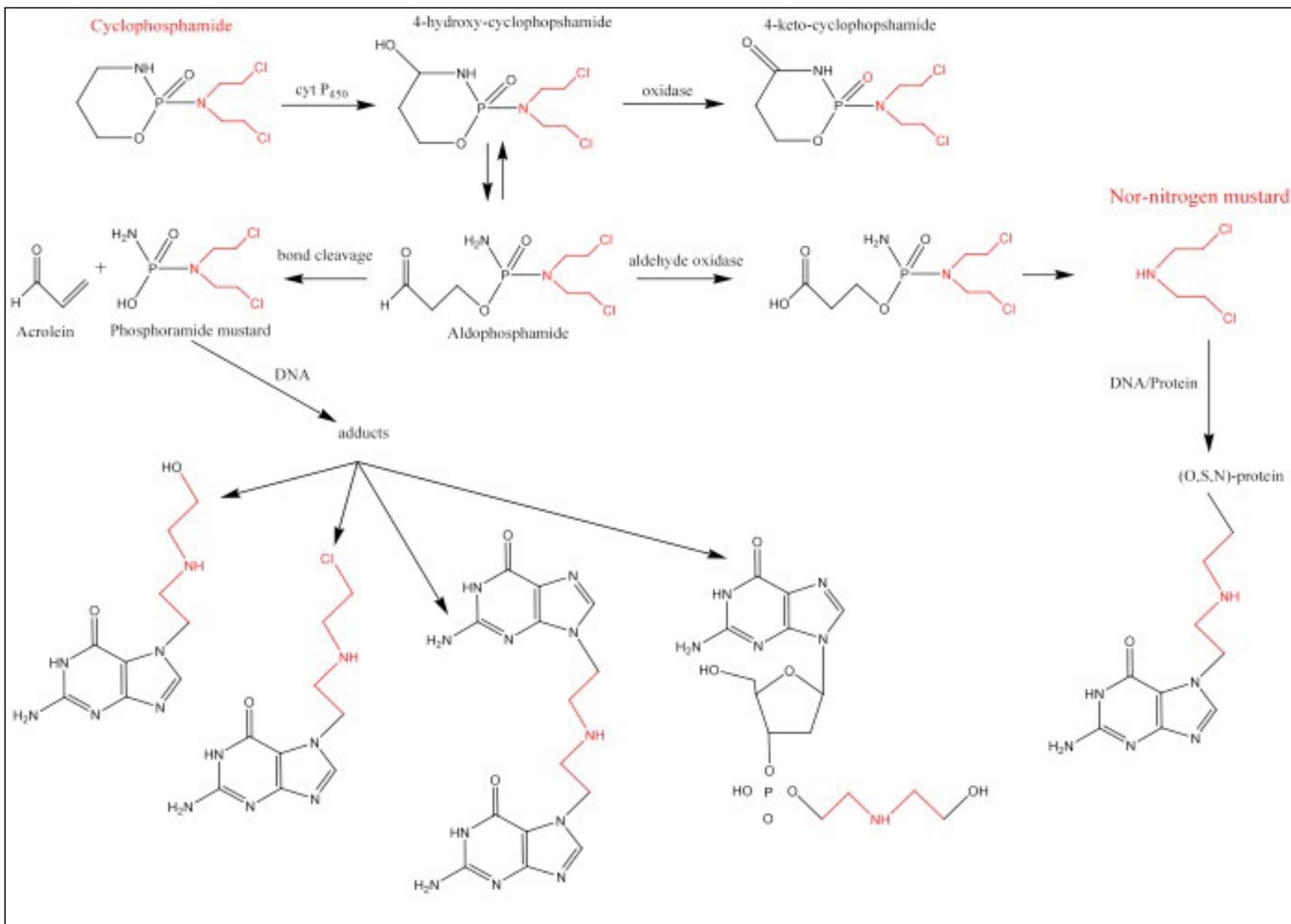


Figure 1.4 Detailed reaction mechanism on the activation of cyclophosphamide and its reactions with DNA

The majority of CP is excreted unchanged from the body through urine (5-25%), sweat and feces (31-66%, after oral dose) [5]. It has an average terminal half-life in the adult body of 6.5hours (1.8-12.4h). In 1981 the International Agency for Research on Cancer (IARC) considered that there is sufficient evidence to place CP in Group 1, as a known human carcinogen [46-47]. Occupational exposure in healthcare settings to cytotoxic drugs has been reported [1,30-31,48-50] and concern has been raised due to potential adverse health effects. Exposure to cyclophosphamide can occur via dermal exposure, inhalation, orally or through punctures of skin with contaminated utensils (such as contaminated needles) [1,45,50-53]. Surface contamination reported included workbenches, the floor between working stations and waste bins, door handles, and storage shelves [54]. Furthermore, contamination on the outside of vials received from manufacturers as well as on storage shelves was reported by Mason et al. in 2003 [55]. Cyclophosphamide has been found in air samples in many hospitals and laundry facilities [18,30,49,55-56].

Nor-nitrogen mustard

Nor-nitrogen mustard, a mustard gas analog, has been considered a Group 2B carcinogen by the IARC since 1985, as there is sufficient evidence of carcinogenicity in animals [57]. Nor-nitrogen mustard is an odourless and colourless liquid with powerful irritating properties. As a solid, it is a white crystalline hydrochloric salt [58]. Nor-nitrogen mustard, along with other nitrogen mustards, was developed as a chemical warfare agent (mustard gas) during World War I. There are no reports of this particular agent being used in warfare [59]. Nor-nitrogen mustard is used as a raw material to produce various cytotoxic/antineoplastic drugs, such as cyclophosphamide [60]. It can be used to treat various cancers, including leukemia and bronchogenic carcinoma, due to its alkylation capabilities. It binds to guanine in DNA with the ethyl chains and inhibits cell reproduction.

A dose-dependent relation has been reported between exposure of human lymphocytes and an increase in chromosomal aberrations and sister chromatid exchanges [60]. Tumours were reported in rats when nor-nitrogen mustard was administered intravenously, while local papillomas were induced when administered topically [59]. Potential occupational exposure is estimated to occur for health professionals or personnel at manufacturing companies [61]. Similar adducts to cyclophosphamide exposure are produced from exposure to nor-nitrogen mustard [62]. However, the yield from nor-nitrogen mustard exposure is reported to be two to three times higher [60].

Methotrexate

Methotrexate (common trade names include: MTX, Trexall, Rheumatrex) is a widely used antimetabolite antineoplastic drug for the treatment of various cancers. This drug was classified by the IARC in 1987 as a Group 3 carcinogen [63]. Other diseases treated with MTX include rheumatoid arthritis or psoriasis. MTX is known to cause chromosomal aberrations in bone-marrow cells and sister chromatid exchange in lymphocytes. Furthermore, the drug is considered mutagenic. Surface contamination has been reported in published literature [64].

Household bleach

Exposure to hypochlorite can lead to irritation, or if exposure persists, it can lead to sensitization [65]. Cleaning with sodium hypochlorite requires its own safety precautions. Toxic fumes (chlorine gas) can evolve from the solution during cleaning. If ammonia is present, chloramines can be liberated from the solution. Furthermore, skin and eye contact can lead to chemical burns.

Objectives

The two main objectives of this research study incorporated chemical applications to eliminate exposure to antineoplastic agents and their by-products. To accomplish this target, the thesis work focused on two separate projects. A task list for each project was determined in order to ensure that the set objectives were accomplished, as follows:

1. Develop and validate an analysis method to identify and quantify nor-nitrogen mustard as a by-product of the decomposition of cyclophosphamide during cleaning with household bleach.
 - i. Development of analysis method for cyclophosphamide and nor-nitrogen mustard.
 - ii. Obtain precise calibration curves for both compounds.
 - iii. Develop extraction procedures for cyclophosphamide and nor-nitrogen mustard from the reaction.
 - iv. Determine the effects of time and bleach volume on decomposition of cyclophosphamide and formation of nor-nitrogen mustard.
 - v. Hypothesize reaction pathways for the interaction between hypochlorite (main ingredient in bleach) and cyclophosphamide.
2. Develop a cleaning agent for cytotoxic agents that removes the drugs by dissolving them rather than by decomposing the agents.
 - i. Determine the suitability of the instrument method (originally developed for cyclophosphamide) for methotrexate.
 - ii. Determine the suitability of the wipe sampling procedures (originally developed for cyclophosphamide) for methotrexate.
 - iii. Determine sampling efficiency and wipe extraction efficiency of the two drugs.
 - iv. Obtain precise calibration curves for both compounds and determine a suitable internal standard.
 - v. Prepare cleaning solutions and determine their efficiency in removing contamination.
 - vi. Determine the most efficient and safe cleaning protocol to ensure complete removal of both drugs in solid form.

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Chapter 2. Potentially carcinogenic by-products arise from cleaning cyclophosphamide with household bleach¹

Introduction

The goal of this study was to confirm the hypothesized hazardous by-product, nor-nitrogen mustard, arising from the cleaning of cyclophosphamide (the most commonly used cytotoxic drug) with household bleach.

Antineoplastic drugs have the ability to cause significant and irreversible cell damage. The same properties that make these chemotherapy drugs so potent in the battle against cancer also raise concerns about adverse health effects due to occupational exposure [1-20]. For cancer patients, the benefits of chemotherapy treatment outweigh the secondary risks; however, exposure in humans to antineoplastic drugs is known to cause adverse health effects including carcinogenic, mutagenic, and teratogenic [1,2,7,9-21] outcomes. Currently there is no recommended exposure limit to these agents [5, 22-24]. Adding to the concern is the potential for adverse cumulative effects from chronic exposure [25]. Workplace exposure to antineoplastic drugs has been well documented for some model antineoplastic drugs (Cyclophosphamide, Ifosfamide, 5-Fluoracil and Methotrexate) with reports coming from Europe and North America [7-21]. Exposure to antineoplastic drugs is not limited to the pharmacy area, but has been detected in oncology wards, administration areas and shipping and receiving departments. Surface contamination, which can potentially lead to dermal and ingestion exposure, has been found on many surfaces including nurses' gloves, hands, door handles and telephones [7-21, 26-

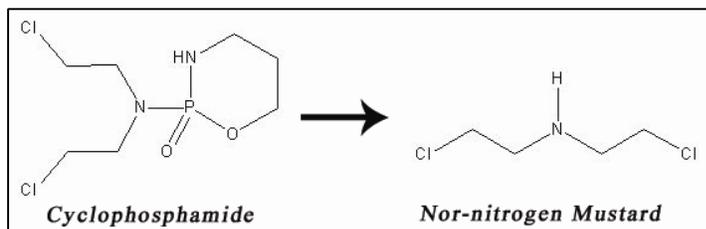


Figure 2.1 Proposed decomposition of cyclophosphamide

28].

Internationally, bleach is one of the most popularly accepted cleaning agents for the removal of cytotoxic drug contamination from surfaces [28-34].

However, the interaction between bleach and some cytotoxic drugs, particularly cyclophosphamide (CP), may lead to the creation of potentially

¹ A version of this chapter will be published. Barzan, C and Chu, W.

harmful by-products. Within the limits of our resources, this project focused on the determination of a potentially hazardous by-product arising from the reaction of cyclophosphamide with household bleach. The main by-product was hypothesized to be nor-nitrogen mustard (2-Chloro-N-(2-chloroethyl)ethanamine), a mustard gas analogue. Nor-nitrogen mustard is an alkylation agent used in various treatments, including chemotherapy (nb: molecule numbers refer to Figures 2.11-2.14). Nor-nitrogen mustard is also used in the manufacturing process of cyclophosphamide [9, 35-37]. Hypotheses could then be applied to suggest all possible reaction pathways in the decomposition of cyclophosphamide by bleach.

Methodology

To confirm the presence of nor-nitrogen mustard, a reliable instrument and sample extraction method was developed to simultaneously identify nor-nitrogen and cyclophosphamide in the presence of hypochlorite (the main ingredient in household bleach). Instrument parameters, chemicals and derivatization procedures were developed and are summarized below and in the result section. To quantify the amount of nor-nitrogen mustard produced during real-world cleaning scenarios, a series of time dependent experiments were carried out. Detailed experimental variables are also summarized below.

(a) Chemicals and reagents

Nor-nitrogen mustard hydrochloride, cyclophosphamide hydrochloride, heptafluorobutyric anhydride (HFBA), pyrene, and tris(2-chloroethyl)amine hydrochloride were all purchased from Sigma-Aldrich. Household bleach, 5.25% sodium hypochlorite, was purchased from a regular grocery store to accurately simulate hospital-cleaning practices for drug-contaminated surfaces. The solvents used: ethyl acetate, ethyl ether and isooctane, were purchased from the university chemical store (Fisher Scientific).

(b) Derivatization and extraction

Two derivatizing agents were tested initially, trifluoroacetic anhydride and heptafluorobutyric anhydride. Stock solutions of cyclophosphamide (5 μ g/ μ L) and nor-nitrogen mustard (5 μ g/ μ L) were prepared in ethyl acetate. An aliquot of 100 μ L of derivatizing agent was added to 100 μ L of stock solutions in ethyl acetate. The resulting mixture was vortexed for 60 seconds and heated to 70°C for 20 minutes. Complete evaporation of the solvent under nitrogen was performed prior to reconstitution in 2000 μ L of isooctane. For sample analysis, the ethyl acetate was blown to dryness and the samples were

reconstituted in 100 μ L of ethyl acetate prior to adding the derivatizing agent. The efficiency of the derivatization was determined to be near 100%, based on spike samples of both compounds.

(c) Calibration graphs

Calibration curves for cyclophosphamide and nor-nitrogen mustard were obtained by dissolving the compounds in ethyl acetate. Pyrene was used as the internal standard because of its stability in the GC as well as the retention time of the molecule is later than the compounds of interest. Serial dilution was used after derivatization with heptafluorobutyric anhydride (HFBA). The ranges for the six-point calibration curves were 0.5ng/ μ L to 100.0ng/ μ L for cyclophosphamide and 0.05ng/ μ L to 5ng/ μ L for nor-nitrogen mustard. The calibration curves had a correlation coefficient of 0.99.

(d) Instrumentation

GC/MS analysis was conducted using an Agilent 6890N gas chromatograph coupled with an Agilent 5973 Mass Spectrometer detector and an on-column injector. The conditions of the instrumentation were developed and are presented in Table 2.2.

(e) Time and volume dependence

In order to determine the time dependence of cyclophosphamide decomposition in household bleach (5.25% sodium hypochlorite), a series of time controlled experiments were conducted to simulate as closely to real-world cleaning situations as possible. Household bleach (4mL) was added to cyclophosphamide (50mg) and the reactions were stopped at different times (0.5, 5, 10, 15, 25 minutes) (see Figure 2.6). The reaction between bleach and cyclophosphamide was also tested with different volumes of household bleach (2, 4, 5 and 6mL). Liquid-liquid extraction with ethyl acetate was carried out after 5 minutes (Figure 2.7). All samples were conducted in triplicate and vortexed for 30 seconds following the addition of household bleach. Aliquots of 100 μ L of the resulting ethyl acetate solution were taken and derivatized. Reconstitution was done in isoctane and analyzed.

Results

The gas chromatogram and mass spectra of the solution containing bleach and cyclophosphamide without derivatization are shown in Figure 2.2. The mass spectrum of the asymmetrical peak with retention time from 8 to 10 minutes confirms the presence of nor-nitrogen mustard (molecular mass 92). A mass spectrum library search confirmed the presence of nor-nitrogen mustard with a 91% match. The mass spectra library is a compilation of known spectra of compounds. The user can determine the

nature of the compound through a match to compound spectra in the mass spectrum library. However, this long retention time, along with the asymmetrical peak shape, prohibits reliable quantification of nor-nitrogen mustard. Therefore, a derivatization method was developed using heptafluorobutyric anhydride (HFBA) as derivatizing agent to improve the reliability of identifying and quantifying nor-nitrogen mustard.

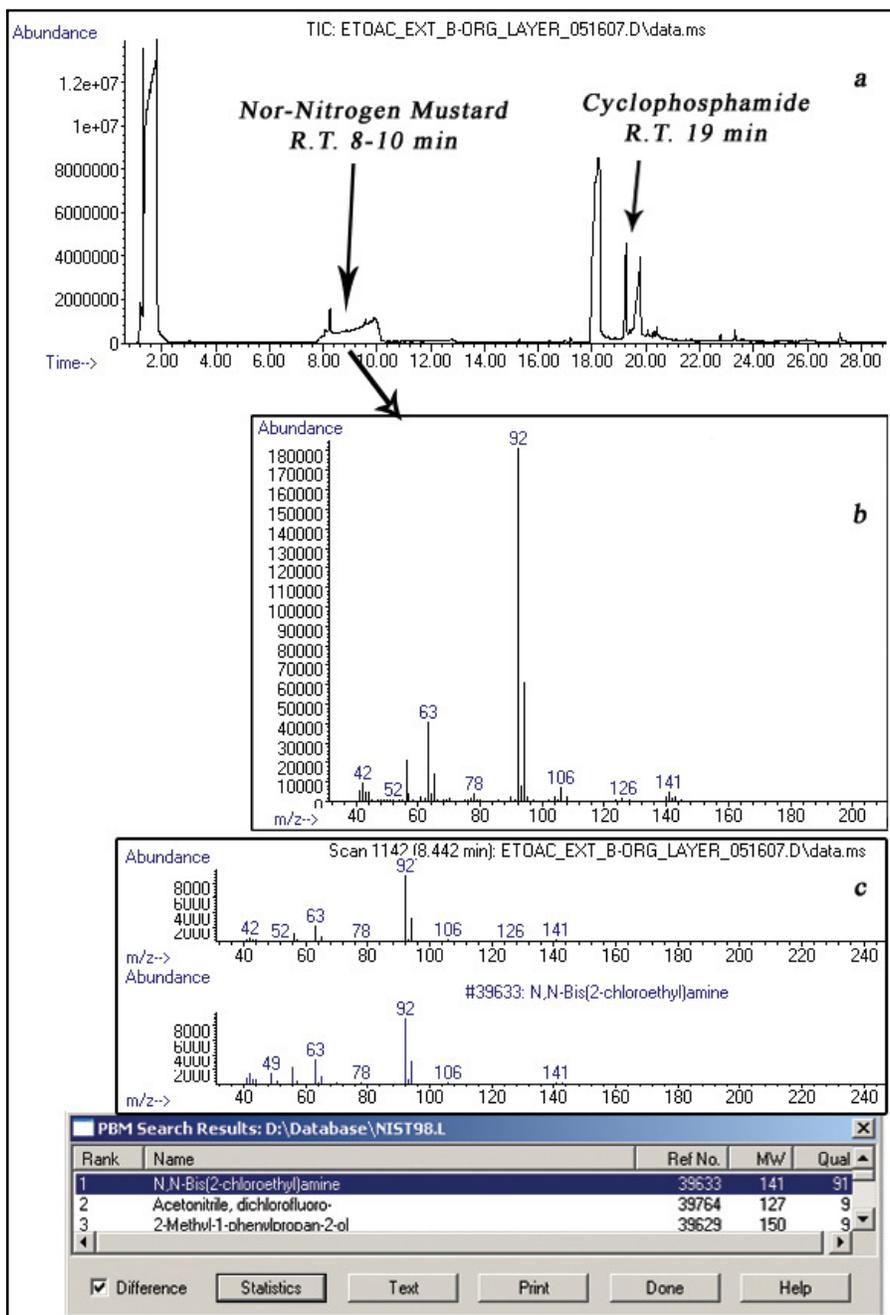


Figure 2.2 Underivatized nor-nitrogen mustard resulting from reaction between cyclophosphamide and 5.25% hypochlorite; **b)** Mass spectrum of unsymmetrical peak between 8 -10 minutes; **c)** Library match of mass spectrum in **b** confirming presence of Nor-nitrogen mustard with a 91% mass spectrum library match

However, the reaction between bleach and cyclophosphamide is complex and yields numerous by-products, most of which can be extracted and detected by the mass spectrometer. As observed in Figure 2.3, after derivatization we were able to obtain a symmetric, sharp nor-nitrogen mustard peak at retention time 10.505 min. This further confirms the presence of nor-nitrogen mustard as one of the by-products arising from the reaction of bleach with cyclophosphamide. However, the baseline noise induced by other by-products interfered with peak quantification in the analysis, thus it was impossible to determine the limits of detection.

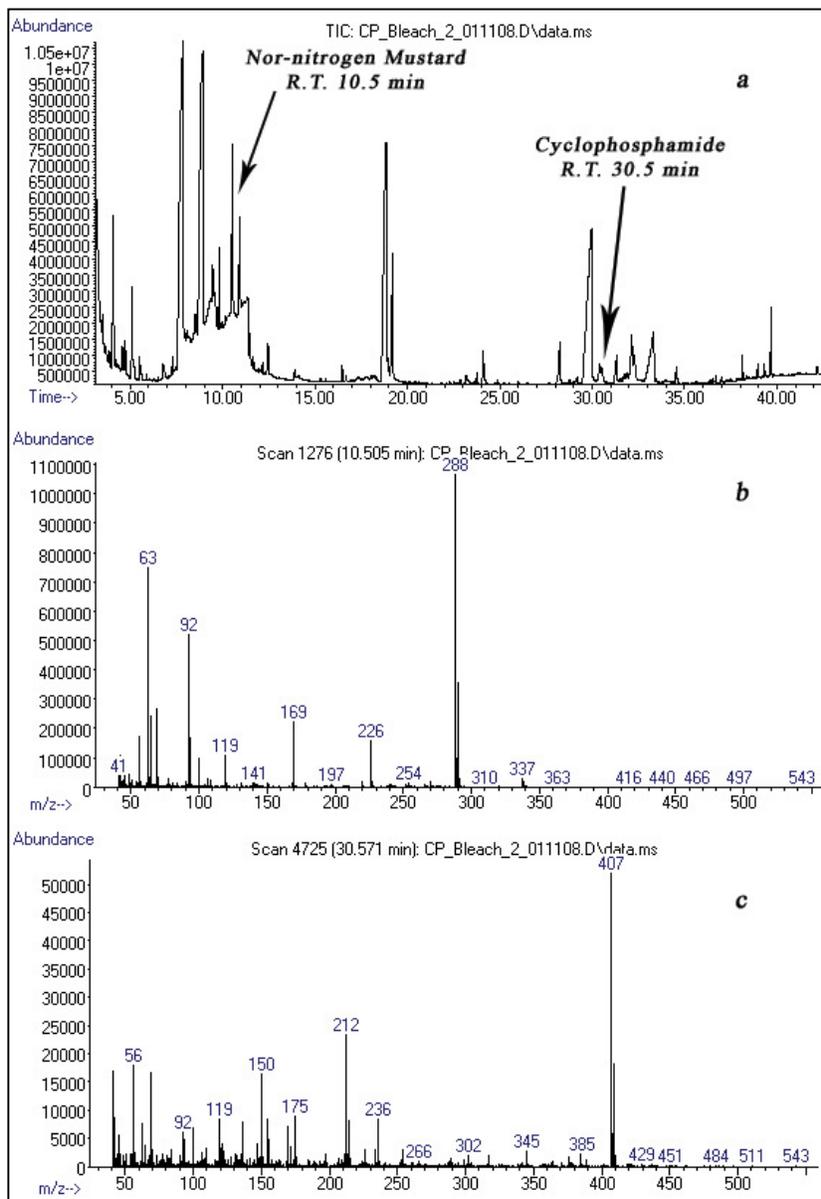


Figure 2.3 Derivatized by-products of 50ug cyclophosphamide reaction with 4 milliliters of 5.25% hypochlorite for 5 minutes; b) Mass spectrum of nor-nitrogen mustard peak at R.T. 10.505 min; c) Mass Spectrum of cyclophosphamide at retention time of 30.571 min

To obtain a quantifiable chromatogram with reliable sensitivity determination, the Single Ion Monitoring Method (SIM) method was used (the instrument parameters are presented in Table 2.2). The chromatogram with improved peak shape and symmetry is shown in Figure 4 with the molecular ion at 288 and fragmentation ions at 226, 169 and 63. The limit of detection was 0.03ng/ μ l.

To monitor the reaction of cyclophosphamide and hypochlorite, similar derivatization and instrumentation methods developed for nor-nitrogen mustard were applied to cyclophosphamide and adjusted for single ion monitoring conditions. As an internal standard, pyrene was added to enhance the reliability of the instrument methodology. Table 2.1 summarizes the ions monitored for derivatized cyclophosphamide, nor-nitrogen mustard and pyrene. The limits of detection were determined for cyclophosphamide to be 0.02 ng/ μ l.

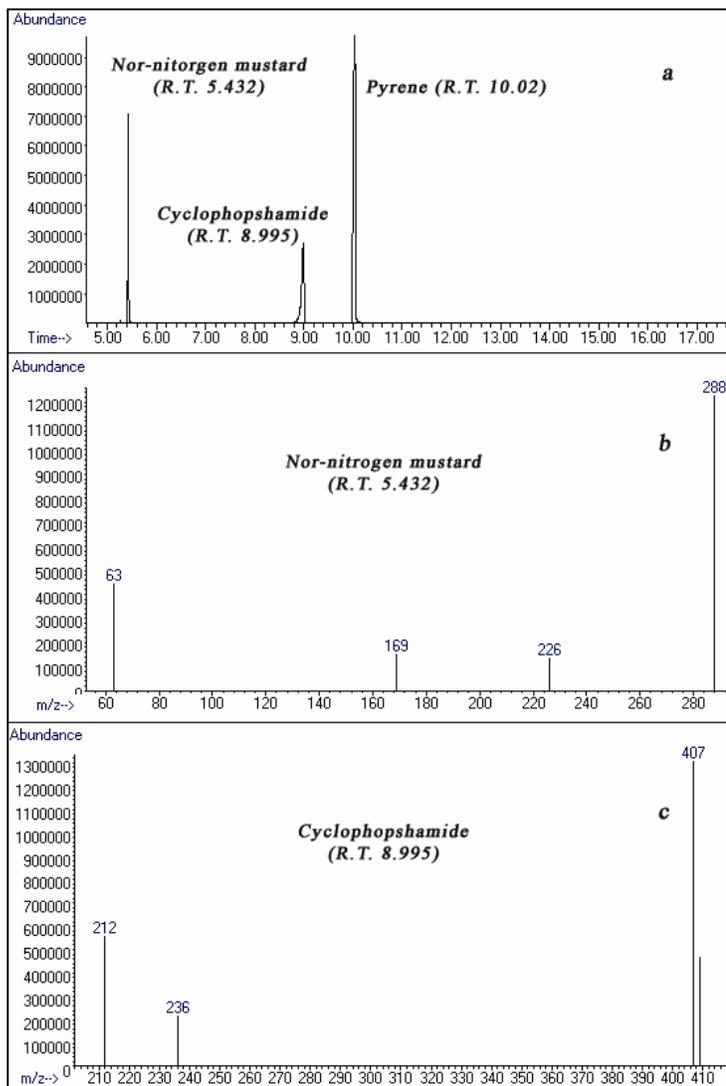


Figure 2.4 a) Single-Ion-Monitoring chromatograph of nor-nitrogen mustard, cyclophosphamide and pyrene; b) Nor-nitrogen mustard SIM spectrum; c) Cyclophosphamide SIM spectrum (R.T. retention time)

Table 2.1 Single ion monitoring

Compound	Ions Monitored
Derivatized Cyclophosphamide	407, 409, 236, 212
Derivatized Nor-nitrogen mustard	288, 226, 169, 63
Pyrene (Internal Standard)	101, 202

The conditions of the gas chromatography and mass spectrometer including column type were developed and are presented in Table 2.2.

Table 2.2 Gas chromatograph mass spectrum conditions

GC Condition	Description
Column Type	DB-5MS, 30m X 0.25mm ID 1.5µm film thickness
Injector temperature	280°C
Column Temperature	Initial temperature 70°C Step 1: 80°C/min to 150°C Step 2: 20°C/min to 200°C Step 3: 10°C/min to 230°C Step 4: hold 1 minute Step 5: 25°C/min to 290°C Step 6: hold 4 minutes, return to 70°C
Gas Carrier and Flow	Helium (1.1mL/min)
Injection Amount	1 µL
Mass Spectrum Detector Temperature	150°C
Mass Spectrum Detection	Single Ion Monitoring

Please see Figure 2.5, the six-point calibration curves were obtained with a correlation coefficient of 0.99 for both cyclophosphamide and nor-nitrogen mustard derivatization with heptafluorobutyric anhydride (HFBA).

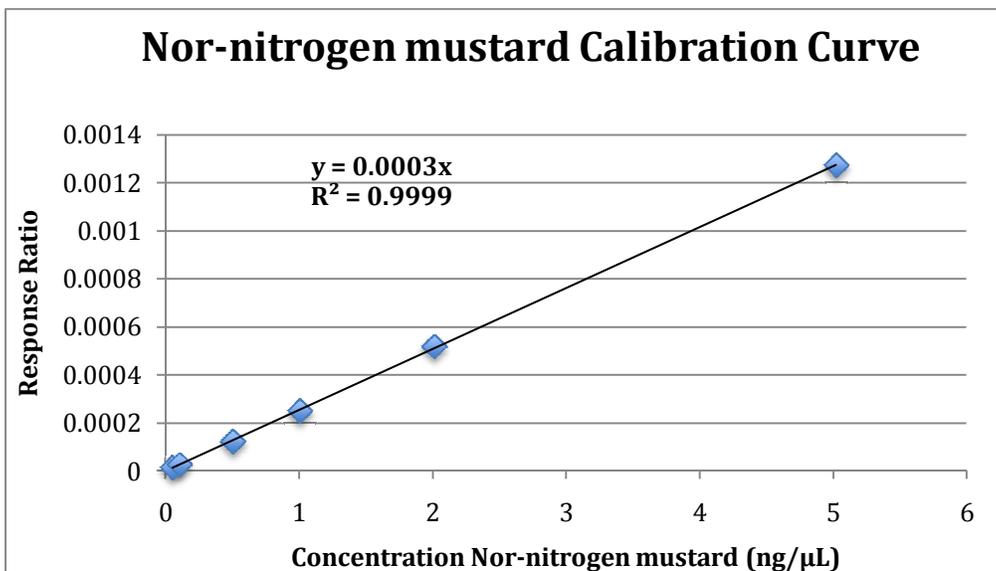
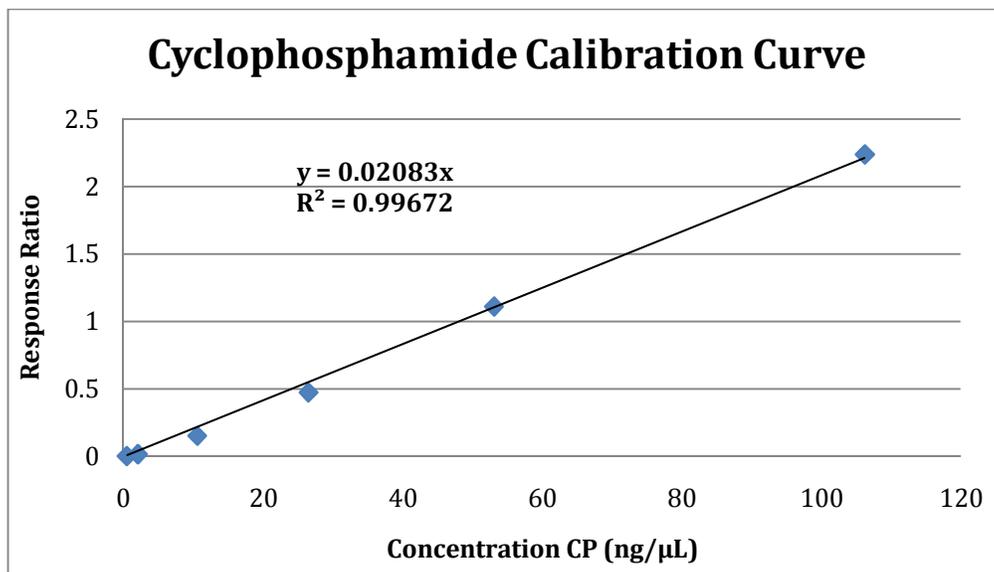


Figure 2.5 Calibration curves of cyclophosphamide (top) and nor-nitrogen mustard (bottom)

In a series of time dependent experiments to find out how much nor-nitrogen is produced within an approximate cleaning scenario, the presence of cyclophosphamide and nor-nitrogen mustard were monitored simultaneously (Figure 2.6, below). The initial concentration of cyclophosphamide was 100ng/μL. About 60% of the drug was decomposed by bleach after 25 minutes; however, the production of nor-nitrogen mustard from the reaction was shown to go through stages of increase and decrease.

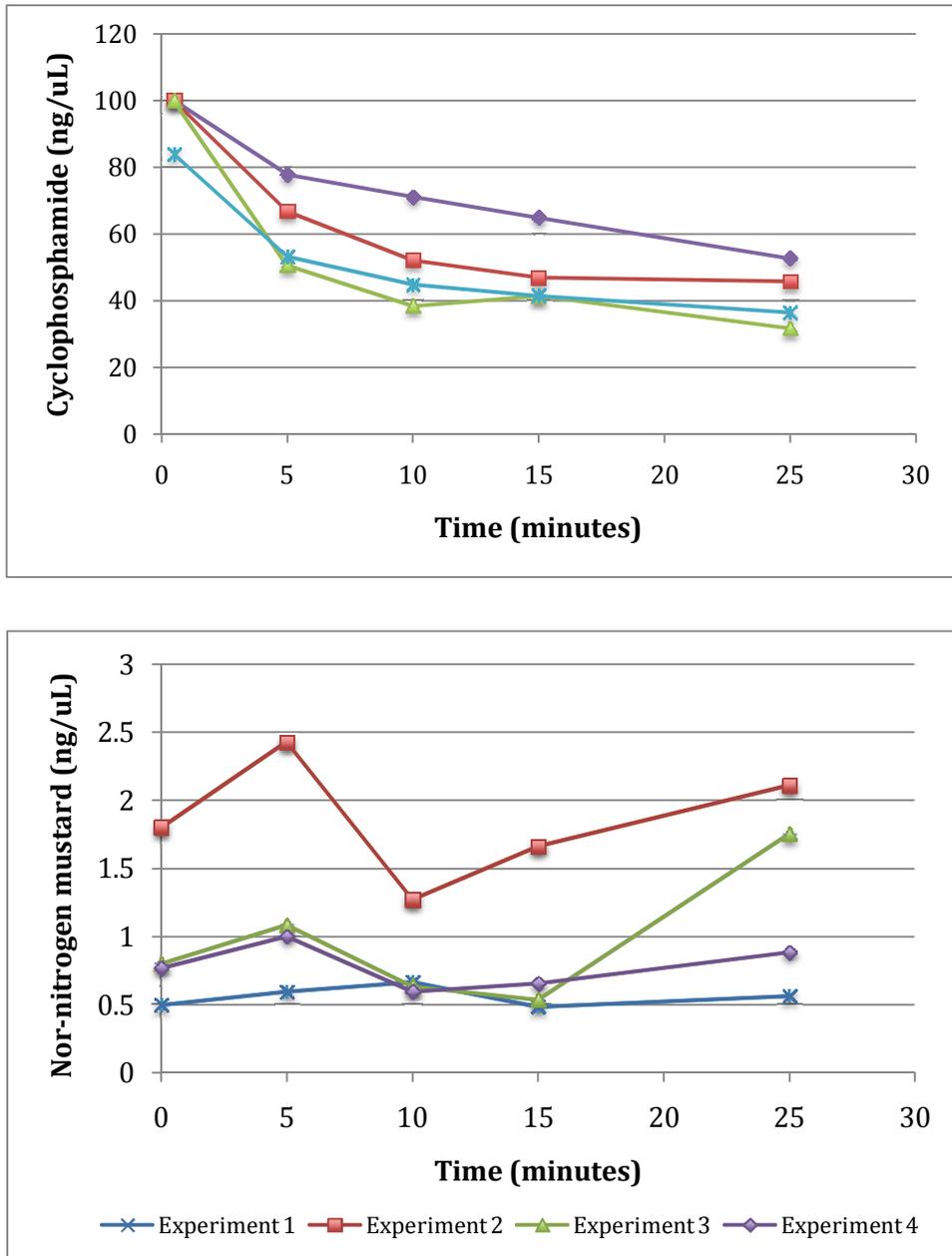


Figure 2.6 Amount of cyclophosphamide (top) and nor-nitrogen mustard (bottom) produced from reaction with household bleach versus time

The decomposition of cyclophosphamide is also dependent on the volume of household bleach (2, 4, 5, 6 mL) used. As the volume of household bleach was varied (Figure 2.7), the production and decomposition of nor-nitrogen mustard was evident.

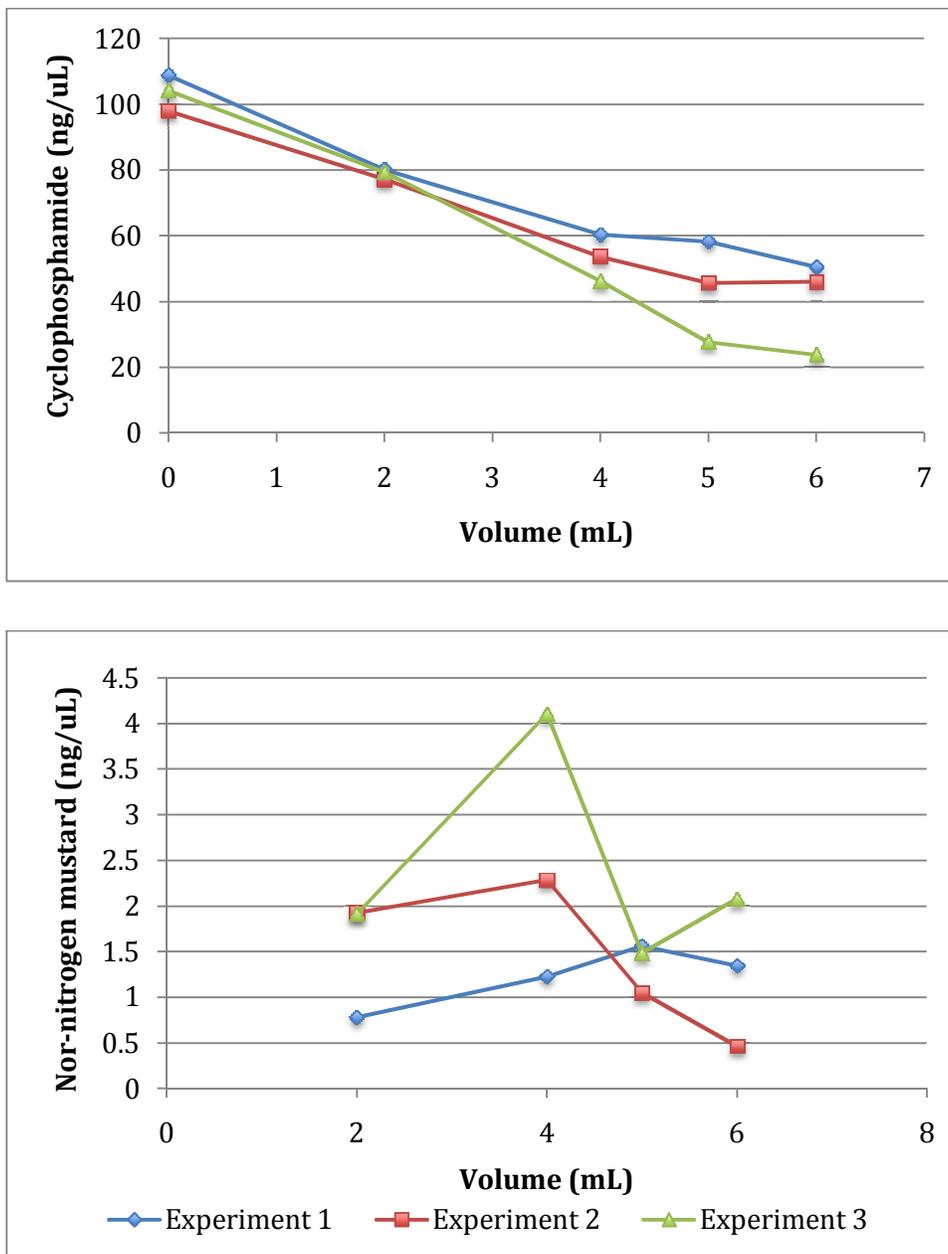


Figure 2.7 Amount of cyclophosphamide (top) and nor-nitrogen mustard (bottom) versus volume of household bleach

Discussion

Derivatization

Figure 2.2 in the results section shows the chromatogram and mass spectrum of underivatized nor-nitrogen mustard. The asymmetrical peak is the result of both thermal instability in the GC and the fact that when dissolved in solution, nor-nitrogen mustard tends to form a highly reactive ethylenediammonium ion intermediate by loss of the chlorine (see Figure 2.8) [36]. This 3-member ring ion will react with any nucleophilic agent, resulting in the formation of various complexes. This reaction mechanism is similar to the alkylation between the nor-nitrogen complex and DNA or protein (refer to Figure 1.1 in Chapter 1) [38-41]. This highly reactive intermediate ion inhibits accurate quantification of nor-nitrogen mustard, as observed by the asymmetrical shape of chromatographic peak in Figure 2.2 and long retention time. Therefore, to quantify the amount of nor-nitrogen mustard, such a compound must be derivatized. Figure 2.8 shows a similar ring structure transformed when underivatized cyclophosphamide is analyzed by GC. The ring is formed due to the thermal instability of cyclophosphamide.

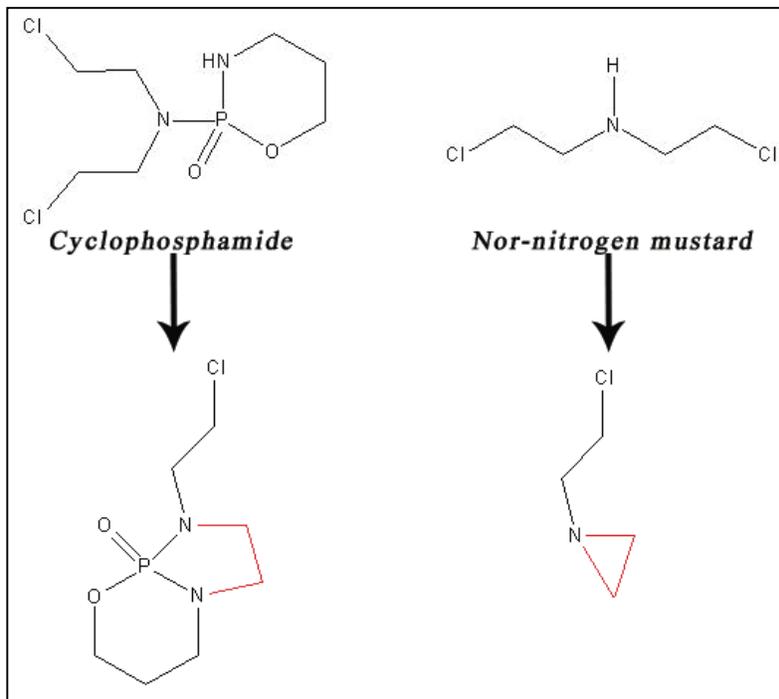


Figure 2.8 Formation of 5-member ring for cyclophosphamide (left); and 3-member ring for nor-nitrogen mustard (right)

The development of the derivatization and instrumentation method for cyclophosphamide and nor-nitrogen mustard took into account that cyclophosphamide and nor-nitrogen mustard would be quantified simultaneously. Therefore, the same derivatizing agent, heptafluorobutyric anhydride (HFBA) was used to react with both compounds in ethyl acetate. An instrumentation method was developed such that both of the derivatized complexes could be identified under the same conditions. An internal standard, pyrene, was added to increase the confidence levels in the quantification of both complexes during the reaction. Fragmentation ions of all three compounds are summarized in Table 2.1 in the results section. Figure 2.9 shows the products for derivatized cyclophosphamide and the nor-nitrogen mustard complex, respectively. Figures 2.3 and 2.4 show the three symmetric and sharp peaks with a much less noisy baseline in the chromatogram. Only from such a chromatogram can confidence be placed in the integration of the compound peak, and detection limits be determined. The limits of detection for cyclophosphamide and nor-nitrogen mustard were determined to be 0.02ng/ μ l and 0.03ng/ μ l, respectively.

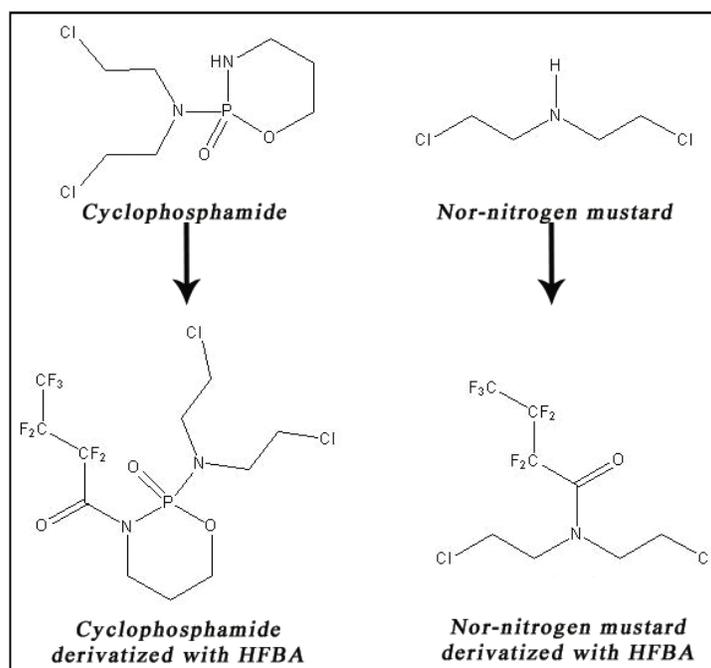


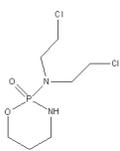
Figure 2.9 Derivatization of cyclophosphamide (left) and nor-nitrogen mustard (right) with heptafluorobutyric anhydride

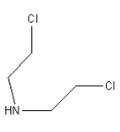
Our hypothesis that nor-nitrogen mustard is formed when household bleach (5.25% hypochlorite) is used to clean cyclophosphamide is further confirmed by examining the reaction chromatogram in Figure 2.3. Metabolized cyclophosphamide combines with the DNA in human cells through the ethyl chains attached to the nitrogen, thus obstructing cell reproduction [38-39]. Furthermore, the same adducts have been identified as a result of exposure to nor-nitrogen mustard, however, the adduct yield from cyclophosphamide is two to three times lower in comparison to nor-nitrogen mustard per amount injected [38]. The cross-linking properties of nor-nitrogen mustard were discovered during World War I when it was produced as a chemical warfare agent together with other nitrogen mustard molecules [36]. The IARC (International Agency for Research on Cancer) currently classifies nor-nitrogen mustard as a Group 2A compound: *reasonably anticipated to be a human carcinogen*. The last review of its potency, however, was conducted in 1985 with minimal review articles [42]. Cyclophosphamide, considered a Group I carcinogen by the IARC, is a widely used alkylation agent [2,3].

Reaction mechanism (please refer to figures 2.10-2.13)

A simplified reaction mechanism is proposed in Figure 2.10 with molecules labeled in sequence.

Hypochlorite OCl^- (the active ingredient in household bleach) is a known oxidizing agent. Therefore,

cyclophosphamide  is thought to decompose by the hypochlorite to create nor-nitrogen

mustard , chlorine gas and two other proposed complexes (not confirmed experimentally).

However, if excess bleach is present after the production of nor-nitrogen mustard, OCl^- will further oxidize nor-nitrogen mustard. Therefore, to understand this multi-step oxidation process, a more complete step-wise multi-path reaction mechanism is proposed in Figures 2.11-2.13.

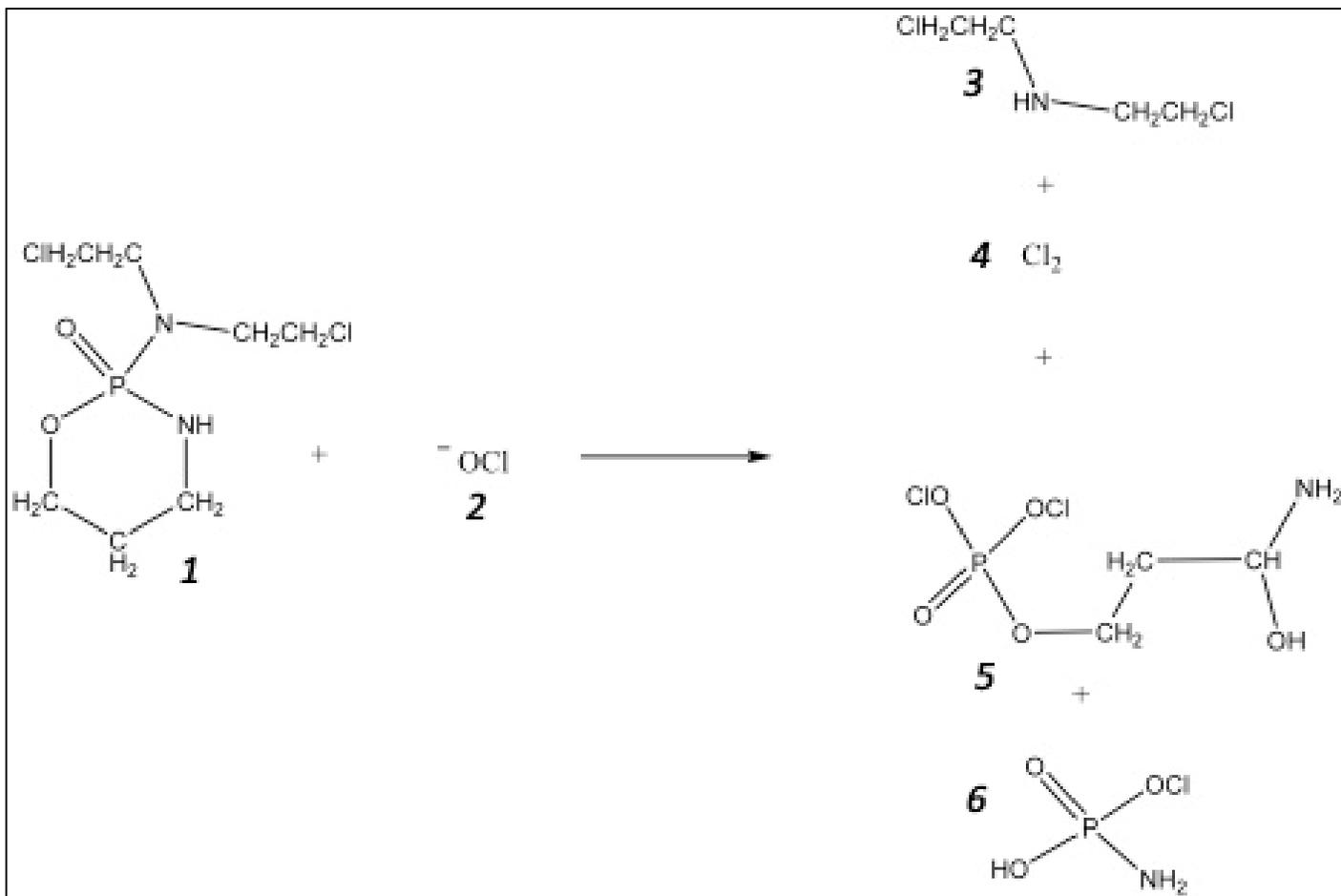


Figure 2.10 Simplified decomposition of cyclophosphamide by hypochlorite

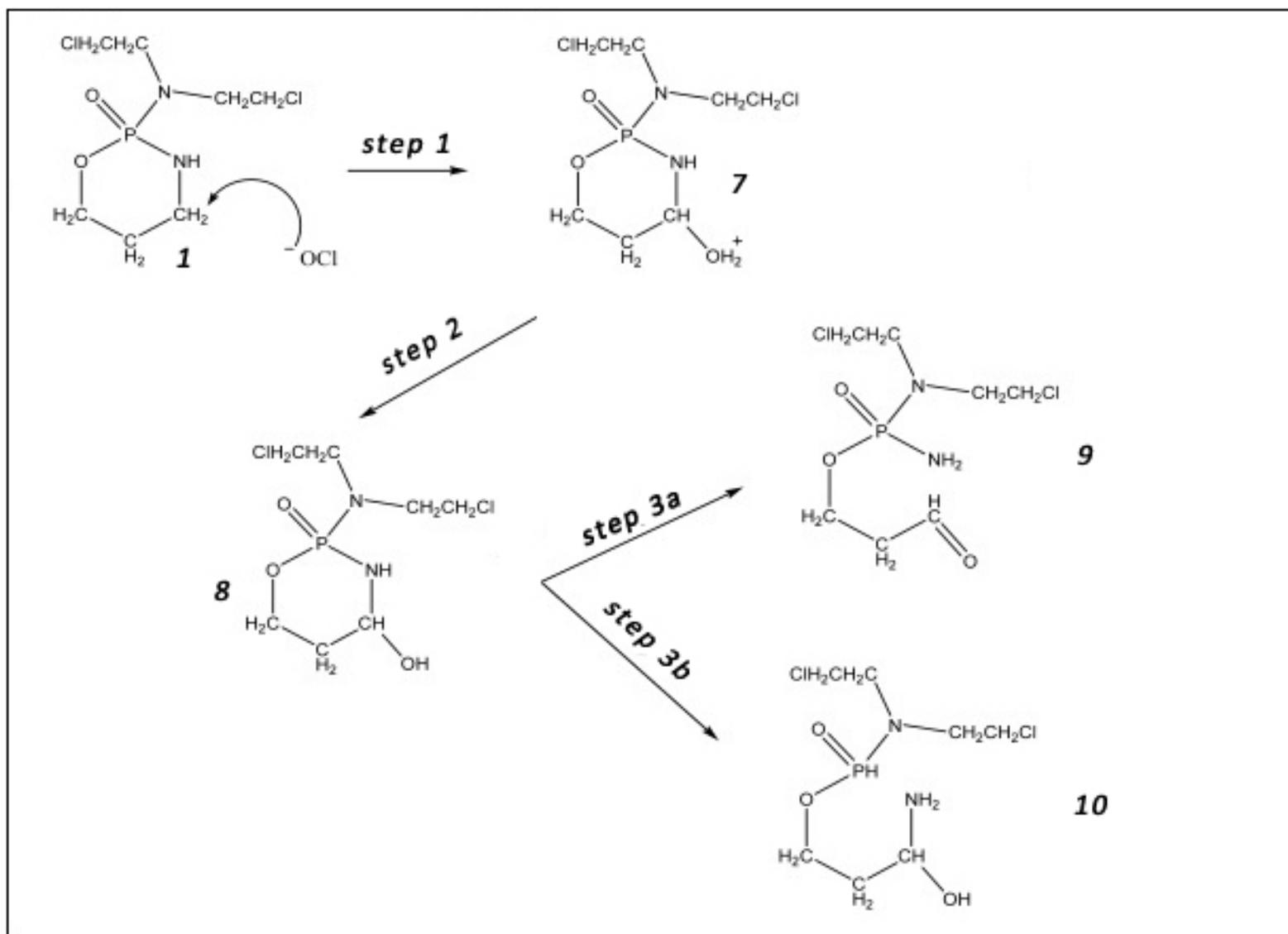


Figure 2.11 Initial oxidation of cyclophosphamide by hypochlorite. Formation of 4-Keto-Cyclophosphamide by oxidation at the C3 carbon

In a multi-step process, hypochlorite from the household bleach is thought to oxidize cyclophosphamide to produce nor-nitrogen mustard (please refer to Figure 2.10). In the first step, OCl^- oxidizes the carbon attached to the amine through a reduction-oxidation process. This allows for the addition of the water molecule to the cyclophosphamide ring. The loss of hydrogen in step 2 forms the 4-Al-Cyclophosphamide (molecule 10). Further oxidation causes ring cleavage to occur after formation of 4-Al-Cyclophosphamide. This ring cleavage can proceed along two different pathways, forming either molecule 9 or 10. Along one of the possible pathways (Figure 2.12, step 3a), further oxidation of the ring carbon and subsequent breaking of the carbon-nitrogen bond creates an NH_2 group (molecule 9). Molecule 9 breaks down further to form acrolein (molecule 12, Figure 2.12) and phosphoramidate mustard (molecule 11, Figure 2.12). Upon further oxidation, nor-nitrogen mustard will be created (molecule 3). Potential step 3b (Figure 2.13) will accelerate the oxidation of phosphorus and cleave the ring at the phosphorus-nitrogen bond. A second oxidation of the phosphorus-nitrogen bond produces nor-nitrogen mustard (molecule 3).

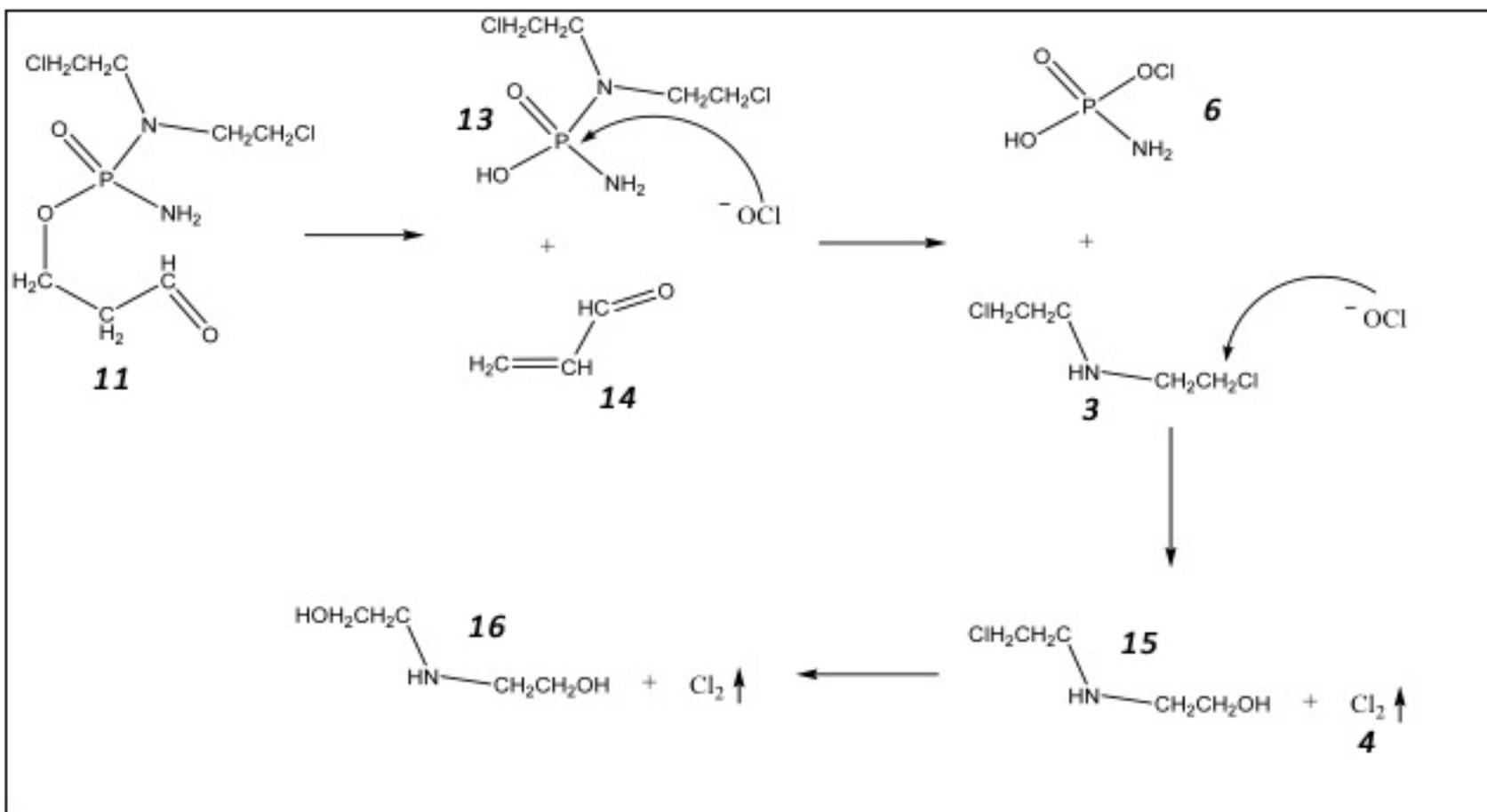


Figure 2.12 Step 3a - Cyclophosphamide ring cleavage by breaking the nitrogen-carbon bond to form NH_2

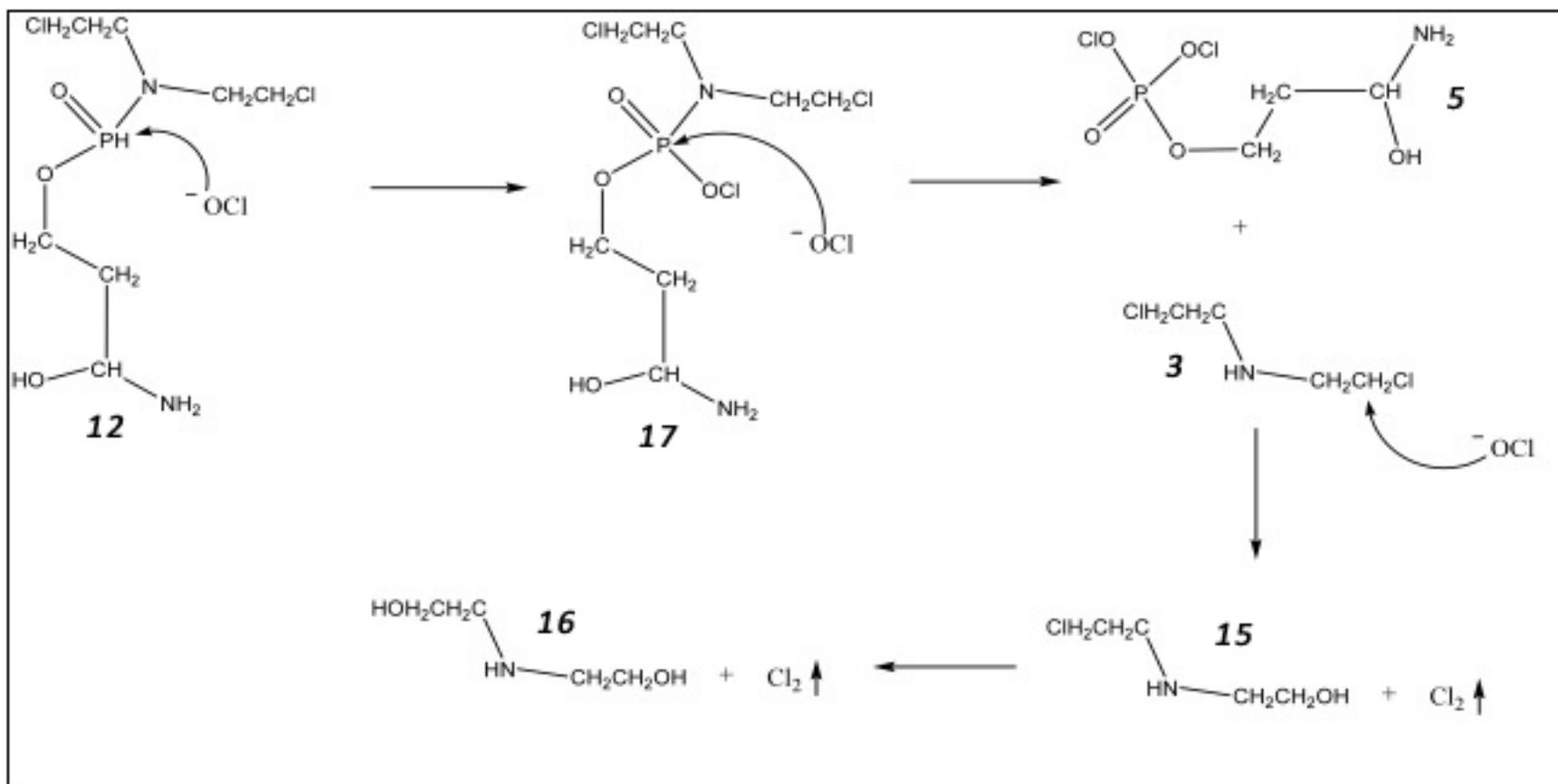


Figure 2.13 Step 3b - Cyclophosphamide ring cleavage by breaking the phosphorus bond to the N₂ nitrogen

Oxidation of the by-products likely continues until either there is little or no hypochlorite left, or there are no other compounds that can be oxidized. Furthermore, oxidation of the ethyl chains in nor-nitrogen mustard is possible in an aqueous-only environment [43]. Chlorine (molecule 4, Figure 2.12) is most likely produced throughout the process, which may favour some particular pathways of reaction over others.

Time dependence of cleaning products

Nor-nitrogen mustard is produced at different steps in the decomposition process. Some nor-nitrogen mustard can be produced directly from cyclophosphamide; however, until a ring cleavage occurs, this is less favoured to occur due to the steric hindrance of the molecule. A competition for oxidation occurs between the by-products of cyclophosphamide, which in turn is evident due to the amount of nor-nitrogen mustard observed based on reaction time (Figure 2.6). As time increases, the amount of nor-nitrogen mustard seems to stay constant but more CP is being decomposed in the process. The volatility of nor-nitrogen mustard must also be taken into consideration, as is evident in the loss of nor-nitrogen mustard which may not be due entirely to decomposition by household bleach. Current cleaning procedures in hospitals include the use of household bleach at the same concentration (5.25% hypochlorite) used in this study. The few studies that have looked at the cleaning efficiency of bleach-containing cleaning agents have failed to study the amount and nature of the by-products created [29, 30, 33-34]. Application of bleach to cyclophosphamide contaminated surfaces will lead to the decomposition of the drug, but also to the creation of by-products.

The proposed potential mechanism also shows that chlorine gas, phosphoramidate mustard, acrolein and other by-products are possibly produced. Confirmation of other by-products was beyond the scope of this project. However, a significant amount of chlorine was detected qualitatively from the reaction in comparison to the amount released from household bleach alone. The volatility of the by-products plays an important role in the efficiency of cleaning procedures. Surface removal of contamination is important but is counterproductive if the procedure itself releases other potentially hazardous products into the air.

Conclusion

Nor-nitrogen mustard, a potentially carcinogenic substance, is identified and quantified as a by-product from the oxidation of cyclophosphamide using bleach. This confirmation was made possible by the successful development of a derivatization method using heptafluorobutyric anhydride (HFBA) to derivatize cyclophosphamide and nor-nitrogen mustard simultaneously. A sensitive instrumentation method utilizing gas chromatography equipped with a mass spectrometer has also been developed to quantify derivatized cyclophosphamide and nor-nitrogen mustard simultaneously. The reliability of the instrument method was further improved by the introduction of a single ion monitoring method. The reaction hypothesis which predicted the production of nor-nitrogen mustard has been proven. This theoretical mechanistic study could be a very powerful tool in the design of new cleaning agents for predicting potential hazardous by-products.

Further work is required to quantitatively determine the levels of other by-products of the reaction, such as chlorine gas, acrolein and phosphoramidate mustard. Moreover, future work is required to develop a cleaning agent that does not decompose carcinogenic substances into other hazardous compounds, potentially exposing workers and the general public.

Limitations

The reactions were performed in capped test tubes inside a controlled laboratory environment and thus do not fully replicate hospital bench-top cleaning procedures. However, this limitation does not negate the fact that decomposition of a carcinogenic substance can be extremely hazardous if the by-products are unknown or uncontrolled. Decomposition of cyclophosphamide will lead to the creation of nor-nitrogen mustard in a real-world setting within 5 minutes after the application of bleach. Variations in the amount created will depend on factors such as surface texture, application mode, physical state of contamination, etc. Although not all by-products were identified experimentally, evidence of the production of nor-nitrogen mustard while cleaning cyclophosphamide with bleach should prove enough to discourage the use of this strong oxidizing agent to remove cytotoxic drugs.

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Chapter 3. Develop a cytotoxic cleaning agent²

Introduction

This project aimed to develop an effective cleaning agent suitable for real world applications in healthcare settings to prevent workers' exposure to cytotoxic drugs.

Occupational exposure to cytotoxic drugs is of great concern to health care workers. Taking into consideration the potency of the drugs, elimination of contamination is the best mode to prevent exposure. Some engineering controls have been introduced to reduced exposure. However, there are no standard cleaning protocols for antineoplastic drugs [1-3]. This may be a reason why contamination with these hazardous drugs is widely detected in healthcare settings worldwide [3-8]. To remove contamination, the cleaning protocols must be designed specific for antineoplastic drugs. An effective cleaning procedure is a key part of any current exposure control plan.

Commonly used cleaning protocols in the province of British

Columbia include using agents such as: household bleach (5.25% hypochlorite), Cavicide®, Phenokil II®, and Chlorhexidine [9]. Despite the fact that these agents are manufactured as disinfectants, they are currently used in antineoplastic contamination cleaning procedures. Three of these cleaners (Cavicide®, Phenokil II®, and Chlorhexidine) have bulky molecules as active ingredient and cannot fully dissolve complex antineoplastic drugs (See Figures 3.1-3.4 for active ingredients of the three cleaning agents) [10-12]. The inability to effectively remove cytotoxic drug contamination of surfaces, which has been shown previously by Chu et al [9], is due to physical and chemical characteristics such as molecular size, complexity and polarity.

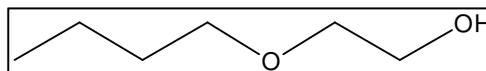


Figure 3.3 Butyl Cellosolve, active ingredient of Cavicide

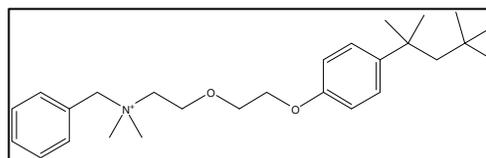


Figure 3.2 Hyamine 1622, active ingredient in Cavicide

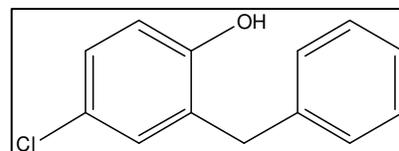


Figure 3.1 O-Benzyl p-Chlorophenol, active ingredient in Phenokil II

² A version of this chapter will submitted for publication and patent. Barzan, C and Chu, W.

While standard cleaning procedures are not official, recommendations on how to deal with cytotoxic drug contamination have been made by agencies such as NIOSH and OSHA [1-2]. They recommend decontamination by oxidation of cytotoxic drugs [2]. Many studies have been conducted to investigate the deactivation of drugs through oxidation. Various oxidizing agents, ranging from sodium hypochlorite to Fenton's reagents have been tried [2, 13-21]. However, these chemicals are rendered useless against unoxidizable drugs. Moreover,

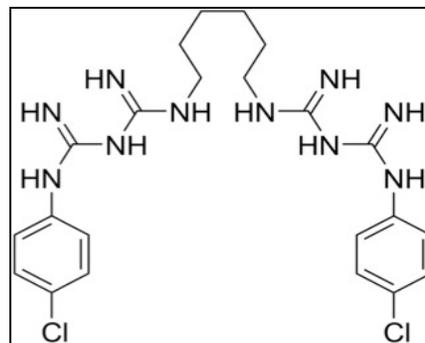


Figure 3.4 Chlorhexidine, main ingredient in Chlorhexeptic

when decomposition by oxidation takes place in open environments, hazardous by-products may be produced and not be contained. For example, decomposition of cyclophosphamide with bleach has been shown to produce nor-nitrogen mustard previously by our research group. Uncontrolled oxidation of potent cytotoxic drugs without full knowledge of the possible by-products should not be recommended. Furthermore, the complete oxidation of some drugs may take as long as 24 hours, but surface cleaning in hospitals occurs rather quickly [15, 16, 18]. Thus, using an oxidizing agent to deactivate cytotoxic drugs can lead to a false sense of security for the workers. Such a situation will lead to an increase in potential exposure.

Furthermore, the only available cleaning agent designed specifically for cytotoxic drugs is Surface Safe®; which consists of a two-wipe process, with one wipe containing 2% sodium hypochlorite and a second wipe containing 1% sodium thiosulphate and 0.9% benzyl alcohol [22]. However, the recommended application of Surface Safe® is limited to drugs that can be oxidized. Since the potential exists for the creation of hazardous by-products when cytotoxic drugs are oxidized, the application of such cleaners may inevitably lead to either the production of hazardous by-products through oxidation, or failure to fully remove unoxidizable contamination. There is, therefore, an urgent need for the development of an efficient cleaning agent for cytotoxic drugs.

Cytotoxic drugs are normally reconstituted in either saline (0.9% sodium chloride) or dextrose (5% dextrose solution). Therefore, all drugs of this kind are hypothesized to have similar solubility properties. The aim of our project was to use this common feature to develop an efficient cleaning agent, which could successfully clean most cytotoxic drugs. Within the limits of the scope of the project, the most commonly used cytotoxic drug, cyclophosphamide, was used to test the efficiency of the cleaning agent. Methotrexate is also a very commonly used cytotoxic drug. Due to its structural differences from

cyclophosphamide, methotrexate was chosen as another ideal candidate to test cleaning efficiency. Furthermore, hypochlorite does not oxidize methotrexate; thus providing a secondary reason for choosing this drug as the second model testing agent.

Methodology

The following experimental procedures outline instrument and extraction methods, as well as sampling and cleaning wipe techniques. Two very different cytotoxic drugs, cyclophosphamide and methotrexate, were used as model drugs to design and test for cleaning efficiency. A cleaning solution was designed from a combination of readily available solvents and alcohols with the aid of salts, such as sodium chloride, to produce a solution with varying polarity. For clarity, moistened Kimwipes used for sampling and extraction are herein referred to as sampling wipes, and moistened Kimwipes used for cleaning are referred to as cleaning wipes. The best cleaning solution tested for both drugs will be used to investigate a suitable cleaning protocol applicable to real-world cleaning situations.

Chemicals and supplies

Cyclophosphamide and Methotrexate were purchased from Sigma-Aldrich Inc.; Deuterium labeled Cyclophosphamide (D4-Cyclophosphamide) was purchased from IIT GMBH. The solvents (methanol, ethanol, isopropanol, and acetonitrile) as well as the inorganic salts (ammonium acetate, sodium chloride, sodium fluoride, and potassium chloride) were supplied by Fisher Scientific and purchased from the University of British Columbia chemical stores; while the KimWipes are manufactured by Kimberly-Clark Inc.

Analysis method

Liquid Chromatography/ tandem Mass Spectrometry (LC/MS/MS) consists of an Agilent 6410 Triple Quadrupole equipped with 1200 Series binary pump, degasser, temperature control auto-sampler, and column compartment. Data acquisition and processing was conducted with Agilent MassHunter (Version B.03.01). An Agilent Zorbax XDB C₁₈ 3.1µm, 4.1x50mm column was used. The mobile phase was composed of 5mM ammonium acetate and methanol; it began at 10% organic and was increased to 90% at an isocratic flow of 0.5mL/min. The column was maintained at 50°C during analysis while the samples were kept at 4°C.

Calibration curves

Calibration curves were prepared in 0.1M-ammonium acetate for both cyclophosphamide and methotrexate using D4-cyclophosphamide as the internal standard. The range of calibration was 0.03 – 15ng/ μ L with R^2 values of 0.99.

Solubility tests

Identical quantities of the tested antineoplastic drugs (cyclophosphamide and methotrexate) were added to test tubes containing equal volumes of the different cleaning solutions. Solubility was observed and determined at 30 seconds, and the results were recorded.

Preparation and extraction of surface sampling wipes

The most effective surface sampling wipes for cytotoxic drugs were determined to be KimWipes by Chu et al [23]. The KimWipes were prepared by adding 1mL of 0.1M-ammonium acetate onto the wipe and kept in capped 20mL vials. An area of 10x10 cm was sampled. Post-sampling, the KimWipes were placed back in the 20mL glass vial with 5.5mL of 0.1M-ammonium acetate. The samples were sonicated for 20 minutes before the extraction of the solution from the wipes through the use of a 10mL syringe. The wipes were placed inside a clean syringe and squeezed until full extraction of solution. Internal standard (50 μ L) is added to 1mL of the resulting solution and analyzed on the LC/MS/MS.

Preparation of cleaning wipes

Moistened KimWipes were prepared prior to the spike experiments and visually inspected to ensure that wipes were completely moistened by the cleaning solution. The cleaning solutions were designed to have various polarities (refer to tables 3.4 to 3.6).

Plate sampling and cleaning procedures

Experiments were carried out on 10x10cm stainless steel plates. Plates were cleaned with a water-methanol mixture (1:1), sonicated for 60 minutes and baked at 180°C over-night between experiments. The drugs were spiked onto the plates at two different levels: low and high (0.455 or 0.92 μ g/cm² for cyclophosphamide and 0.495 or 0.99 μ g/cm² for methotrexate, respectively). Solution, wipe and plate spike controls were prepared during each experiment. Solution spike controls were used to determine the extraction efficiency for the wipe and surface sampling controls (details follow). Each set of experiments was conducted in triplicate. Furthermore, to ensure the plates did not contain any

contamination between experiments, a random set of two plates were chosen as blank plate controls. The blank plate control plates were not spiked with any drug and were sampled following protocol.

Cytotoxic drug stock solutions were first prepared by dissolving them in 0.1M Ammonium Acetate (450ng/ μ L cyclophosphamide and 490ng/ μ L methotrexate). The same volume of drug stock solution was spiked into 0.1M Ammonium Acetate as solution controls and onto the KimWipes as a wipe controls. The same volume of drug stock solution was spiked onto a stainless steel plate and allowed to dry. Air-dry procedure prevents the dissolved solution from interfering with the removal of the drugs from the plates in later steps. With the exception of the spike plate control samples, all other plates were then cleaned with moistened cleaning wipes using one wipe per plate. Post-cleaning, all of the plates were sampled following our established wipe sampling protocol in a systematic fashion [24]. Briefly: wipes were folded in half and the plate was wiped in a horizontal direction, taking care to ensure that the entire surface was wiped and the same force applied between the different plates. The wipes were then unfolded and the unexposed side of the wipe was used to wipe the same plate vertically. The used wipe was then rolled up and placed into an extraction tube. From this step onward, wipe spike and solution spike samples were treated the same way as all other samples following the established procedures for the preparation and extraction of surface wipes. Following the one-wipe cleaning technique, a multiple-wipe cleaning technique was also tested on the most efficient cleaning agents for both drugs.

Data processing

Quality control of the extraction efficiency was performed by comparing surface control recovery to solution and wipe control samples for each batch of tests as well as by examining the linearity and inter-day data of the quality control samples (see Table 3.1). Drug removal efficiency (cleaning efficiency) was determined by comparing levels of drugs left on the plates to surface sampling controls (which were not cleaned with any solutions).

Results

Quality control

The representative calibration curves for cyclophosphamide and methotrexate, which were used to quantify cleaning efficiency, are plotted in Figure 5. As can be seen from the graphs, both calibrations have the required linearity, with an R^2 value of 0.99.

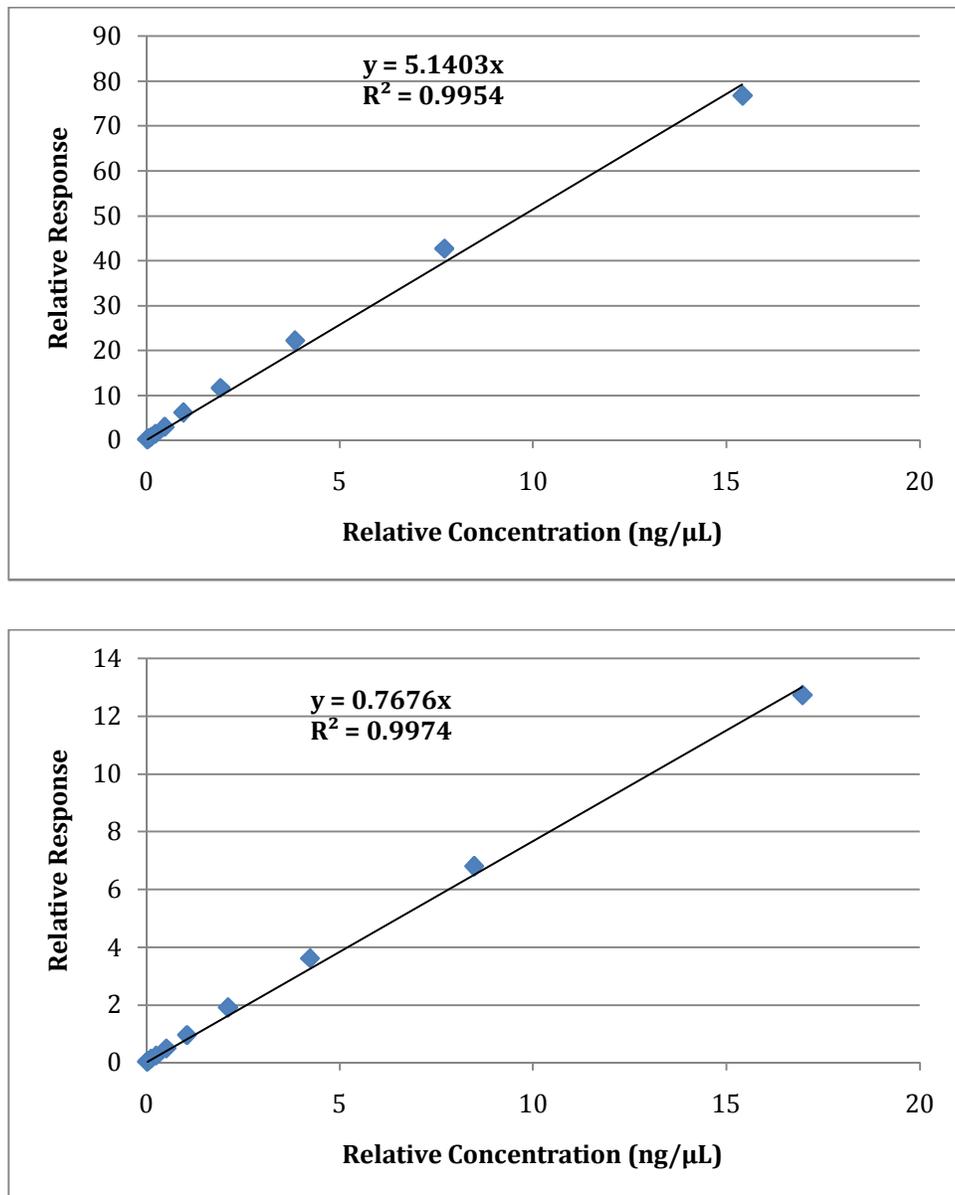


Figure 3.5 Methotrexate(top) and cyclophosphamide (bottom) 10-point calibration curves

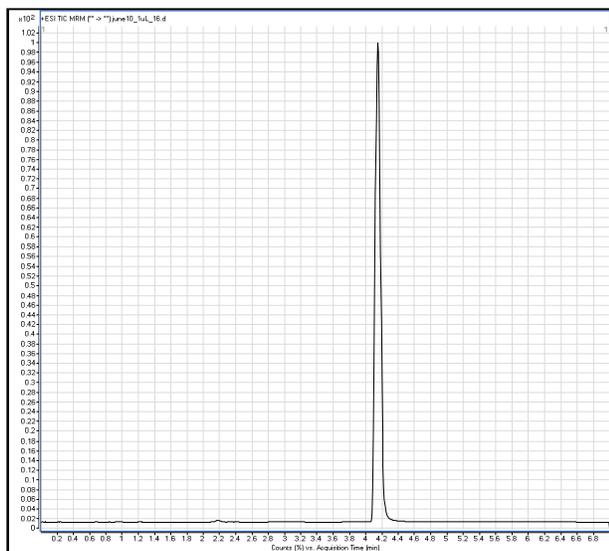


Figure 3.6 Chromatograph of Plate Blank sampled with Internal Standard Peak (with relative response on vertical axis; retention time of peak on horizontal axis)

Blank plate controls were analyzed with each batch. A representative chromatograph of a blank plate control is shown in Figure 3.6; which contains only the internal standard peak.

The spike solution controls samples used to obtain the average response over the batch analysis had concentrations of 45.5 or 92 $\mu\text{g}/\text{cm}^2$ for CP (0.45 or 0.92 $\mu\text{g}/\text{cm}^2$), and 49.5 or 99 $\mu\text{g}/\text{plate}$ for MTX (0.495 or 0.99 $\mu\text{g}/\text{cm}^2$), for the low and high spikes respectively. Table 3.1 shows the percentage of control spike recovery comparing to the same levels of drugs during each batch analysis for all experiments.

Table 3.1 Representative quality control samples average response through batch-analysis and variation between different experimental days for low and high control spikes (0.455 or 0.92 $\mu\text{g}/\text{cm}^2$ for cyclophosphamide and 0.495 or 0.99 $\mu\text{g}/\text{cm}^2$ for methotrexate, respectively)

	Cyclophosphamide			Methotrexate		
	Average (%)	SD	Range	Average (%)	SD	Range
Low	98.87	0.88	98.0 - 100.3	92.52	7.38	84.8 - 101.7
High	98.02	0.92	96.9 - 99.3	90.43	6.79	81.3 - 95.9
Low	98.32	1.59	95.7 - 100.2	111.32	4.51	104.9 - 119.1
High	99.14	1.56	97.2 - 101.8	109.07	4.68	102.4 - 117.2
Low	99.22	0.69	97.8 - 99.9	92.42	4.46	83.9 - 96.6
High	98.86	0.83	97.8 - 99.9	91.2	5.32	83.1 - 96.9
Low	99.77	2.84	95.3 - 102.8	102.27	3.07	98.1 - 105.9
High	98.9	1.06	97.2 - 100.7	101.07	1.86	97.2 - 103.4

SD - Standard Deviation

The three types of controls: solution spike, wipe spike and plate spike were prepared and used to determine extraction efficiency for each set of experiments. Extraction efficiencies are reported in Table 3.2. The efficiency is calculated by comparing the recovery from the wipe and surface spike to that of the solution.

Table 3.2 Wipe sampling efficiency for cyclophosphamide and methotrexate

Agent (replicate number)	Extraction Efficiency from Wipe (%)			Surface Efficiency (%)		
	Average Efficiency (%)	SD	Range	Average Efficiency (%)	SD	Range
Cyclophosphamide (66)	98.37	4.26	89.7-103.9	97.36	5.76	89.5-109.7
Methotrexate (42)	83.00	9.68	66.2-95.5	64.33	7.11	57.2-76.0

SD - Standard Deviation

Cleaning efficiency

Solubility tests

Table 3.3 Drug solubility test results

Solute	Cyclophosphamide	Methotrexate
Ethanol	S	S
Methanol	S	S
Isopropanol	S	S
Saline	S	S
Cavicide	SS	SS
Phenokil II®	IN	IN
Chlorhexidine	IN	IN

S = soluble within 30 seconds; SS = slightly soluble longer than 30 seconds, within 5 minutes; IN= insoluble

Single alcohol solution: The cytotoxic drugs (cyclophosphamide and methotrexate) were spiked as low and high contamination. The cleaning efficiency of each of the three alcohols at concentrations of 50% using a single wipe is reported in Table 3.4. Cleaning efficiency (% removal) was calculated by comparing the recovery of the sample plate (cleaned surface) to the control plate (without cleaning) spiked with the same level of drugs. As can be seen in Table 3.4, 50% methanol alone can remove up to 93% of

cyclophosphamide; however, only about 77% of methotrexate was removed with a 10.45% standard deviation of 24 replicates.

Table 3.4 Removal efficiency of cyclophosphamide and methotrexate with methanol, ethanol and isopropanol

Cleaning Agent	Cyclophosphamide				Methotrexate			
	%Removal	SD	Range	Replicates	%Removal	SD	Range	Replicates
Methanol 50%	93.47	2.11	90.3-97.4	24	77.46	10.45	63.4–92.6	24
Ethanol 50%	92.39	1.71	89.7-95.0	18	70.41	13.55	49.5–88.7	18
Isopropanol 50%	88.53	4.97	80.6-93.0	18	70.02	4.51	62.1-75.8	18

SD - Standard Deviation

Mixed methanol solution: Pure alcohols, such as methanol and ethanol, which seem somewhat effective in removing cyclophosphamide (~93%), were then mixed with the same salt solutions (such as saline) used in typical drug preparation processes or administration procedures to potentially aid the cleaning efficiency. Cleaning solutions were prepared using clean methanol in combination with the following: clean acetonitrile, ammonium acetate (0.1M), sodium and potassium chloride (0.9% by weight), and sodium fluoride (2.1% by weight). Results are summarized in Table 3.5. As can be seen from the data, except in the case of methanol mixed with acetonitrile and saline (which reduced cleaning effectiveness for cyclophosphamide), all other combinations increased the cleaning efficiency. The results varied in surface contamination removal from 84% to 96.2% for cyclophosphamide and 38% to 98.4% for methotrexate with a single wipe. The mixture of methanol: 0.9% potassium chloride: 0.1M ammonium acetate (1:1:1) was found to be most effective for cleaning cyclophosphamide (96.2%), with a 91.2% removal efficiency with methotrexate. However, more than 98% methotrexate can be removed by a mixture of methanol: 2.1% sodium fluoride: 0.1M ammonium acetate (2:1:2). This solution has 95.9% removal efficiency when applied to cyclophosphamide.

Mixed ethanol solution: (Please refer to Table 3.6) When ethanol was mixed with the same type and concentration of salts and solvents, the cleaning efficiency in the removal of both drugs increased except when mixed with saline at one to one ratio. Over 90% cleaning efficiency for cyclophosphamide was obtained with almost all solutions tested. However for methotrexate, improved cleaning efficiency

was obtained by using a mixture of alcohol and salt-based solution. Sodium fluoride addition provided the highest removal (>97%) for methotrexate; while for cyclophosphamide the removal was >94% after one wipe.

Table 3.5 Methanol based cleaning agent combinations; arranged in increasing efficiency of cyclophosphamide removal

Cleaning Agent	Replicates	Cyclophosphamide			Methotrexate		
		%Removal	SD	Range	%Removal	SD	Range
Methanol: Acetonitrile (1:1)	6	84.06	3.59	81.5 – 86.6	38.25	0.92	37.6 – 38.9
Methanol: 0.9% Sodium Chloride (1:1)	24	91.56	3.92	84.4 – 95.8	80.42	10.31	65.4 – 95.0
50% Methanol	24	93.47	2.11	90.3 – 97.4	77.46	10.45	63.4 – 92.6
Methanol: 0.9% Sodium Chloride: Acetonitrile (1:1:1)	18	94.57	1.67	91.4 – 96.0	87.23	12.42	62.8 – 95.4
Methanol: 0.1M Ammonium Acetate (1:1)	12	94.78	0.70	94.0 – 95.5	84.88	8.33	73.3 – 92.2
Methanol: 0.9% Sodium Chloride: 0.1M Ammonium Acetate (1:1:1)	18	95.43	1.46	93.5 – 97.3	90.76	6.44	78.6 – 94.9
Methanol: 2.1% Sodium Fluoride: 0.1M Ammonium Acetate (1:1:1)	12	95.78	0.85	94.8 – 96.8	96.84	0.56	96.2 – 97.5
Methanol: 2.1% Sodium Fluoride: 0.1M Ammonium Acetate (1:2:1)	12	95.85	0.38	95.5 – 96.3	97.62	2.06	95.6 – 99.9
Methanol: 2.1% Sodium Fluoride: 0.1M Ammonium Acetate (2:1:2)	12	95.99	0.62	95.5 – 96.9	98.41	1.78	96.8 – 100.0
Methanol: 0.9% Potassium Chloride: 0.1M Ammonium Acetate (1:1:1)	12	96.20	0.78	95.1 – 96.8	91.24	2.49	88.5 – 94.6

Table 3.6 Ethanol based cleaning agent combinations; arranged in increasing efficiency of cyclophosphamide removal

Cleaning Agent	Replicates	Cyclophosphamide			Methotrexate		
		%Removal	SD	Replicates	%Removal	SD	Replicates
Ethanol: 0.9% Sodium Chloride (1:1)	18	91.24	4.08	84.8 – 93.9	73.52	14.62	55.8 – 89.3
50% Ethanol	18	92.39	1.71	89.7 – 95.0	70.41	13.55	49.5 – 88.7
Ethanol : 0.1M Ammonium Acetate (1:1)	12	93.32	2.23	91.4 – 95.9	81.47	4.34	75.1 – 84.7
Ethanol: 2.1% Sodium Fluoride: 0.1M Ammonium Acetate (2:1:2)	12	94.03	1.34	92.2 – 95.6	96.91	1.63	95.9 – 99.3
Ethanol: 0.9% Potassium Chloride: 0.1M Ammonium Acetate (1:1:1)	12	94.73	0.78	93.6 – 95.3	94.00	3.42	89.8 – 98.1
Ethanol: 2.1% Sodium Fluoride: 0.1M Ammonium Acetate (1:2:1)	12	95.05	0.80	94.2 – 96.1	95.94	2.65	92.6 – 98.4
Ethanol: 2.1% Sodium Fluoride: 0.1M Ammonium Acetate (1:1:1)	12	95.43	1.09	94.3 – 96.7	95.90	1.26	94.3 – 97.2
Ethanol: 0.9% Sodium Chloride: 0.1M Ammonium Acetate (1:1:1)	18	95.82	0.94	94.4 – 96.8	92.35	4.24	86.8 – 95.6
Ethanol: 0.9% Sodium Chloride: Acetonitrile (1:1:1)	12	95.89	0.53	95.2 – 96.5	94.17	1.25	92.6 – 95.6

SD - Standard Deviation

Cleaning protocol investigation

A cleaning protocol was designed and investigated for easy implementation in real-world cleaning situations. Moistened Kimwipes with methanol: 2.1% sodium fluoride: 0.1M ammonium acetate (2:1:2) were used to clean both drugs using a dual-wipe cleaning technique followed by a 70% isopropanol wipe. Results are summarized in Table 3.7. It can be seen from the data that both wipes removed more than 99% of cyclophosphamide and methotrexate. The additional wipe using readily available 70% isopropanol leaves no trace of either drug on the surface.

Table 3.7 Cleaning efficiency of dual-wipes and 70% isopropanol

Drug	Methanol: 2.1% Sodium Fluoride : 0.1M Ammonium Acetate (2:1:2)				70% Isopropanol	
	Wipe 1		Wipe 2		Wipe 3	
	%Removal	SD	%Removal	SD	%Removal	SD
Cyclophosphamide	95.99	0.622	99.74	0.141	>99.99	N/A
Methotrexate	98.41	1.776	99.74	0.085	>99.99	N/A

SD - Standard Deviation

Discussion and conclusion

Quality control

The linearity of the calibration curves and the consistency of the inter-day quality control spikes provide confidence in the cleaning data produced by our analytical instrument and extraction methods. Although the extraction efficiency for methotrexate ranged from 64% to 83% for surface sampling wipes and cleaning wipes, respectively, the extraction efficiency for CP was about 98% for both. The instrumentation and extraction methodology was optimized for the extraction and analysis of CP but not for methotrexate. An extraction method for methotrexate could potentially be developed separately from that of CP; however, with limited resources and the fact that cleaning efficiency was the main objective, the extraction method developed and optimized for CP was also used for methotrexate. The consistency of the extraction efficiency of both surface sampling and cleaning wipes assures that the

extraction method can be used to calculate cleaning effectiveness. Plates were cleaned between experiments and the data confirmed that no drug residue was present prior to each set of spike experiments.

Cleaning efficiency

The concept behind the design of cleaning agents is to produce an agent that will dissolve drugs effectively. The ability to dissolve the contaminant is crucial to its complete removal, especially when oxidation or reduction is deemed to cause potentially hazardous by-products. If the cleaning agent does not dissolve the contaminant, then drugs on the contaminated surface may simply be pushed around and not be picked up by the cleaning wipe. Moreover, in real-world cleaning situations cleaning procedures take place rather quickly. Therefore, the reaction time between the cleaner and the drug must be of short duration. Ideally the cleaning agent should dissolve the drug within seconds. It is common practice for cytotoxic drugs to be reconstituted in an aqueous based solution with either sodium chloride or sugar added prior to being administered to patients. Therefore, the design principle for a cleaning agent should take advantage of such known salts and solvents mixing in with alcohols that are readily available and commonly applied to manipulate various cytotoxic drugs.

Testing only two drugs may seem like a limitation, but the differences in molecular structure, shape and polarity (with different functional groups) between cyclophosphamide and methotrexate provide an adequate indication of how cleaning agents would perform on other drugs. Both drugs have been used as model drugs for their respective class of antineoplastic agents. This includes mode of activity in the body, chemical structure and shape, chemical composition and function groups available. As mentioned earlier, a main common characteristic of all cytotoxic drugs is their ability to be assimilated and active inside the human body. This implies that although different in molecular structure, their solubility is the common feature to be exploited in cleaning.

Cleaning was performed after the spiked drug solution was fully evaporated, thus leaving the drug residues in solid form on the surface. This is a very important factor, because if the contamination is in solution on the surface, the removal of contamination is determined by the absorbency efficiency of the wipes. The active ingredients are not needed as the drugs are already suspended in the solution itself; thus, removal of solution will remove contamination. In every set of experiments, there was always a set of control plates, spiked at the high and low level, which did not get cleaned with any solution. The

amount of drugs recovered from this control plate was compared to that of the post-cleaning plates. This formula determined the cleaning efficiency.

Although all alcohols tested (methanol, ethanol and isopropanol) cleaned cyclophosphamide with around 90% removal efficiency, this alcohol group does not seem to work on methotrexate. Hence, it is likely that there is a very limited application for such single alcohol based cleaning agents. The addition of sodium fluoride and ammonium acetate enhanced the cleaning efficiency for both drugs significantly. After one wipe, this mixture removed drug contamination by 99.99% for MTX and 97% for CP. Furthermore, a dual-wipe cleaning procedure removed 99.99%+ of both compounds. The use of 70% isopropanol is a standard procedure in hospitals to sterilize surfaces. This additional procedure was proven to completely eliminate any traces of cyclophosphamide and methotrexate on a stainless steel surface. This “mixture” cleaning agent is composed of ingredients commonly found in hospital settings and the concentrations used in the cleaning agent were not hazardous to human health.

To eliminate cytotoxic drug exposure to healthcare workers, surface contamination of drugs must be removed and surface cleanliness monitored. A mixture of solvents with varying polarity to dissolve drugs of different structure and functional group has proven to be very effective. The cleaning agent developed has been shown to remove cyclophosphamide and methotrexate contamination completely. Using the solubility concept to design cleaning agents eliminates the potential hazards associated with by-products produced by oxidation or reduction of drugs. We hypothesize that this “mixture” cleaning agent could be effective with other types of hazardous antineoplastic drugs. The development of such cleaning agents would prove to be beneficial in reducing surface contamination and hence reduce workers’ exposure to cytotoxic drugs. Our developed instrumentation and extraction methods to sample surface drug residues could also be used to monitor surface cleanliness and ensure a safe working environment. The incidence rate of cancer has been growing every year; therefore, prevention by reducing occupational exposure to carcinogenic substances would prove to have the valuable benefits of improved quality of life and reduced treatment costs.

Limitations

Within the limitations of our resources, cleaning effectiveness could only be tested on stainless steel surfaces. However, stainless steel surfaces (inside biological safety cabinets, for example) are the most common type of surface in areas where drugs are prepared. They are also the surface type most commonly contaminated and where spills occur. Other surface materials such as linoleum, carpets or

wood do exist in hospitals. Due to the specific nature of the developed cleaning agent, contamination can be dissolved readily and absorbed into the wipe. However, to fully understand how different types of surfaces change the effectiveness of the cleaning agent, further testing needs to be conducted.

Experiments were carried out on high levels of drug contamination. Experiments with trace levels of drug spiking may need to be carried out as well. However, the high spike drug levels in this project, in combination with our sensitive analytical method, provided the opportunity to notice a 500-fold removal of contamination. High contamination levels are expected to be found in hospital settings following either a spill or continuous contamination of surfaces. The ability to completely remove contamination at high levels from surfaces will help prevent exposure to antineoplastic drugs in healthcare settings.

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Chapter 4. General discussion and conclusion

To eliminate surface contamination of cytotoxic drugs and hence reduce occupational exposure to these hazardous drugs, two main objectives were investigated for this research work. The first research objective was to hypothesize and confirm the potential hazardous by-products arising from cleaning cyclophosphamide with bleach. The second objective was to design and create an effective cleaning agent to remove surface contamination of cyclophosphamide and methotrexate.

This chapter will discuss the proposed hypothesis and conclude experimental findings as well as summarize strengths and limitations. The outcomes of these two experimental projects have compelling implications for healthcare working environments and policy changes. Key messages from both chapters are summarized below.

Key messages

A reliable analytical method to identify and quantify cyclophosphamide and nor-nitrogen mustard simultaneously

To confirm the presence of nor-nitrogen mustard from the interaction between hypochlorite and cyclophosphamide, a sensitive method was developed using gas chromatography equipped with a mass spectrometer. It was found that both cyclophosphamide and nor-nitrogen mustard are too thermally unstable to survive the high temperature oven in the chromatograph. Both molecules are hypothesized to form a highly reactive ethylenediammonium ion intermediate, which was evidenced from the asymmetrical broad chromatographic peak. More thermally stable compounds were created from derivatizing cyclophosphamide and nor-nitrogen mustard with heptafluorobutyric anhydride. A single-ion-monitoring method was developed for both derivatized compounds in GC/MS, since the reaction chromatogram shows multiple reaction by-products interfere with the two main compounds.

The successful development of the analytical method with a quantifiable chromatographic peak is crucial to the identification and quantification of the cytotoxic drugs and their by-products.

Nor-nitrogen mustard produced from decomposition of cyclophosphamide

After the development of the analysis method for the simultaneous identification of cyclophosphamide and nor-nitrogen mustard, nor-nitrogen mustard was confirmed to be one of the by-products arising from cleaning cyclophosphamide with bleach. This mustard compound alkylates with cell DNA and inhibits reproduction at a higher yield than does cyclophosphamide [1]. Exposure to nor-nitrogen mustard could occur during cleaning or after, as the molecule can escape into the surrounding environment during surface cleaning.

Oxidation of cyclophosphamide with household bleach, although recommended by the NIOSH, creates hazardous by-products [2-3]. Besides the cleaning of biological safety cabinets, most cleaning (including floors and bench top surfaces) occurs in open space, which may lead to additional exposure to hazardous by-products as well as to cytotoxic drugs

The application of reaction hypothesis

The reaction hypothesis process for predicting hazardous by-products has proven to be a powerful tool in the prediction of nor-nitrogen mustard. This method could potentially be used to hypothesize hazardous cleaning by-products and used in the design of cleaning agents. A detailed reaction mechanism for the interaction between cyclophosphamide and hypochlorite to produce nor-nitrogen mustard and other by-products was proposed.

Ineffective cleaning of cytotoxic drug contamination with oxidation

Experimental data in Chapter 2 showed that 60% of cyclophosphamide was oxidized by bleach after 25 minutes. It is likely that residue drugs are still left on the contaminated surfaces after cleaning with bleach. This false sense of security may cause higher exposure to workers who assume surfaces are clean and thus fail to wear gloves, for example. Furthermore, hypochlorite is ineffective against drugs that cannot be oxidized. Therefore, the practice of using hypochlorite-based cleaning agents is an ineffective practice against cytotoxic contamination.

Policy concerning decontamination of cytotoxic drugs needs revision

Current cleaning procedures in British Columbia healthcare facilities require revision. As mentioned in Chapters 2 and 3, the current cleaning agents used are oxidizing agents and disinfectants [4]. Disinfectants are not able to fully dissolve and remove the contamination; oxidizers, such as hypochlorite, react with the contamination but create dangerous by-products. Cleaning procedures

should be revised which accurately account for the cleaning effectiveness of the agents used as well as any potential interaction with the contamination.

Development of an effective cleaning agent and protocol

The solubility properties common to antineoplastic drugs can be used to effectively remove contamination and thus create a safer workplace. The use of a cleaning agent that is able to dissolve contamination enables its absorption by cleaning wipes. This protocol is safer than on-surface decomposition as it removes contamination without creating by-products. Furthermore, the contamination can then be deactivated in a closed environment (i.e. closed vessel). This practice will contain any by-products formed, and thus protect workers from exposure.

Strengths and limitations

Limitations

Controlled reaction

A primary limitation of the reaction analysis between cyclophosphamide and household bleach is the test-tube environment in which the reactions were carried out. The reaction between the two compounds was conducted in closed cap test tubes, and therefore did not fully replicate hospital conditions where the actual interaction between the two compounds occurs on surfaces in open-air environments. It is difficult to establish how this limitation would affect the amount of nor-nitrogen mustard produced as a by-product. However, it is thought that since the reaction was conducted in the closed lab situation, the decomposition of nor-nitrogen mustard was actually more effective during the experiments. In an open space situation, nor-nitrogen mustard could escape and enter the working environment, potentially increasing exposure to workers.

Extraction efficiency determined from water

Extraction efficiency for both compounds was determined by extraction from water solutions. Water will interact with both compounds if enough time is allowed for the reaction. However, the extraction from water for both compounds was conducted immediately after the compounds were dissolved, thus avoiding oxidation. Furthermore, water is the main ingredient in household bleach. Therefore,

extraction from water is deemed a good substitution since the hypochlorite is a more potent oxidizing agent and will interact with the compounds immediately.

Analysis of a single by-product

Analysis of only one by-product from the interaction of hypochlorite with cyclophosphamide can be seen as a limitation to our conclusions. The presence of other by-products was detected but their nature was not analyzed. The oxidation of cyclophosphamide yields numerous by-products, which can change as the reaction progresses over time. During the thesis work, the identification of other by-products was considered; however, due to lack of funding and the need for specialized analytical equipment, it was decided that the amount of work added was beyond the scope of the thesis. Identifying further compounds would have exponentially increased the time required for this project and was beyond its initial scope. Proof of the presence of one hazardous by-product arising from the decomposition of a carcinogenic substance using a commonly used cleaning agent was deemed to provide enough evidence that current cleaning protocols are not adequate. An increase in time and funding to arrive at the same conclusion was determined to be an unnecessary waste of resources.

Cleaning experiments conducted on steel plates

The efficiency of cleaning solutions prepared was tested on stainless steel plates. Although stainless steel is the most common drug preparation surface, in the real world many other types of surfaces are found in hospitals including: concrete, linoleum, hardwood floors, and so on. The efficiency of the cleaning agent has been proven to work when the surface is hard and does not permit drugs to penetrate into the surface. However, the cleaning efficiency of our developed cleaning agent is achieved because the drugs are dissolved immediately. Therefore, if the contamination can be rapidly dissolved by the cleaning agent and extracted by some means, our cleaning agent would be the preferred choice over one that cannot rapidly dissolve drugs [Ref Chapter 3]. That being said, it is strongly recommended that the cleaning efficiency should be further tested on different surfaces.

Extraction efficiency of methotrexate

The extraction efficiency of methotrexate, 64.5%, can be considered a limitation of the stated cleaning efficiency (>99.7%). However, the percentage removal of methotrexate was obtained by comparing the amount left on the plates after cleaning with the detected amounts of spiked methotrexate on the

control plates. The procedure was repeated with each cleaning test to provide consistency between different experiments. The efficiency of the cleaning agent is relative to the amount detected on the control plates. The percentage recovery is determined relative to control-spiked solutions with known amounts of the drug. Therefore the percentage cleaning efficiency reported included the percentage surface removal efficiency.

Cleaning agent development tested only two cytotoxic agents

The removal efficiency of the cleaning agent was tested on two model drugs, cyclophosphamide and methotrexate. Both cytotoxic drugs have been used as model drugs in the literature for exposure determination as well as decomposition potential. Furthermore, they are widely used agents and exposure to them is known to cause adverse health outcomes. Each of the two agents is representative of its respective antineoplastic classification in regards to mode of action. The assumption that they will behave similarly to other classes of antineoplastic drugs when cleaned is a limitation to the project. Further research into the cleaning efficiency of other antineoplastic drugs is recommended. However, a cost versus information analysis had to be addressed. The testing of all antineoplastic drugs was not practical, especially within the scope and funding of this project.

Strengths

Specificity

The analysis of cyclophosphamide decomposition was specifically developed for the analysis of nor-nitrogen mustard. This aspect of analysis allowed for sensitive detection of the compound of interest. The presence of nor-nitrogen mustard as a by-product arising from the interaction with bleach provides evidence that decomposition of carcinogenic substances should not be conducted in an open environment. Removal of contamination needs to take into account the potential release of by-products and any traces of these by-products left behind on surfaces.

Solubility properties

There are a limited number of studies that have tested cleaning efficiency of antineoplastic drugs. However, all studies were focused on decomposition of the compounds. This is the first study where solubility of the drugs was taken into account and used to clean contamination. Reaction between the cleaning agent and the contamination was undesirable, as cleaning will occur in open environments.

Therefore, efficient cleaning by immediate solubility was considered a safe route for contamination removal. The ability to dissolve contamination immediately allows for rapid absorption onto wipe material. Cleaning a compound from a surface with a solution that fails to dissolve it is ineffective and only smears the contamination around. Such a cleaning method is only as efficient as the force and duration of scrubbing with cleaning wipes, and will not likely produce a clean environment.

Cleaning protocol

The cleaning efficiency was determined relative to the level detected on the spike plates. Spike controls (plate, wipe and solution) were conducted with each experiment. This repeated protocol provided information about consistency between experimental days. Furthermore, the cleaning efficiency was standardized so that it could be compared between different cleaning agents and experimental days.

Current knowledge and introduction of new research conclusions

Current common cleaning procedures in hospitals include the use of hypochlorite to remove cytotoxic agent contamination. This practice is recommended by NIOSH [2]; however, standard cleaning procedures have yet to be established. Furthermore, the use of antibacterial cleaning agents is also practiced in British Columbian hospitals [4-5].

The conclusions drawn from this project suggest that current cleaning protocols may not be adequate in safely dealing with cytotoxic contamination. The by-products produced with hypochlorite contradict the current prevalent opinion that on-surface, open environment deactivation through decomposition of cytotoxic drugs is a safe practice. We have shown the production of nor-nitrogen mustard as a by-product from the reaction. Furthermore, the decomposition of cyclophosphamide is not immediate. Cleaning is usually conducted quickly, thus a cleaning agent should remove contamination immediately.

We have developed an efficient cleaning agent for cytotoxic drugs that does not decompose the contamination. The cleaning agent works by fully dissolving the contamination and thus enabling quick absorption into cleaning wipes. The development of our cleaning agent should provide a safe alternative to current ineffective cleaning agents.

Overall significance

Providing a possibility to improve cleaning efficiency of cytotoxic contamination in hospital settings summarizes the overall significance of this thesis project. The first project provides evidence that open environment decomposition (i.e. oxidation) of carcinogenic substances should not be a recommended practice. Accordingly, this evidence must be taken into consideration in order to create a safer occupational environment and reduce the potential exposure to hazardous compounds. After providing evidence that current cleaning protocols are inefficient, it was very important to provide information to the healthcare sector on the best method to remove contamination of dangerous agents. Overall, the significance of this project is improved safety for healthcare employees and the general public through elimination of potential exposure to antineoplastic agents.

Potential application of research findings

The confirmation of nor-nitrogen mustard as a by-product of cleaning cyclophosphamide with household bleach will influence cleaning policymaking. It is expected that the use of household bleach as a whole will be re-evaluated wherever the potential for antineoplastic agents exists. Furthermore, the efficiency and safety of other oxidizing agents in the removal of carcinogenic contamination in open environments should be re-evaluated in light of our results. Further investigation is necessary to determine the safety of oxidizing carcinogenic compounds in open space.

The developed cleaning agent, as part of the developed cleaning protocol, will be marketed as a cytotoxic drug-cleaning agent and cleaning procedure. Further experiments are expected to confirm its efficiency with other drugs.

Future perspective

Test other cytotoxic drugs

We have confirmed high cleaning efficiency of the developed cleaning agent for cyclophosphamide and methotrexate. The two drugs are representative of their respective antineoplastic class. The cleaning agent developed by us is expected to be more effective than any other cleaning agent currently used in healthcare setting. However, testing of other drugs should be conducted systematically to confirm this.

Test other types of surface

Stainless steel surfaces are a commonly found in hospital pharmacy departments. However, other surfaces include linoleum and carpets. The cleaning efficiency of our cleaning agents is based on the ability to dissolve the drugs fast. However, the effectiveness on surfaces such as linoleum, carpets or wood should be tested.

Cleaning protocol adjustments

Future work should also include revision of current cleaning procedures in hospitals to ensure adequate changes are conducted. Hazardous by-products, if produced, reduce the efficiency of the cleaning agents used. Therefore adding to the potential occupational exposure due to the assumption that the environment is clean and safe. An addition to this, the cleaning protocols should be adjusted to include a requirement of standard procedure prior to choosing an adequate cleaning agent for the appropriate contamination type.

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