# An Environmental and Biological Study of Occupational Exposure to Cyclophosphamide in the Pharmacy of a Japanese Community Hospital Designated for the Treatment of Cancer

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Cancer treatment in Japan is considered to be very progressive in the use of antineoplastic agents. Pharmacists are required to compound more antineoplastic preparations recently and thus are at risk for exposure to antineoplastic agents. In Japan, healthcare professionals have recognized the need for protection, but there have been few reports of environmental contamination or occupational exposure to antineoplastic agents. In the present study, urine samples over 24 hr from compounding pharmacists and wipe samples from the compounding room were collected to analyze levels of cyclophosphamide (CP). CP was detected in urine samples from all 4 pharmacists (mean = 165.3 ng/24 hr) and for all wipe samples of the compounding room. From the results of these tests, the standard operating procedure (SOP) of the pharmacy was revised as a protective measure. The tests were then repeated and CP was detected again in the urine of all 4 compounding pharmacists, though the mean CP level was reduced from 165.3 to 47.4 ng/24 hr after the revision of the SOP. Although there was no correlation between the amount of CP compounded and the CP levels in urine initially, the 2 values were significantly correlated after the revision of the SOP ($R^2$ = 0.87).

**Key words** —— antineoplastic agent, exposure, cyclophosphamide, urine sample, wipe sample

## INTRODUCTION

The Japanese Society of Hospital Pharmacists (JSHP) published its "Guidelines for Handling Antineoplastic Agents in Hospitals" in 1991, which was updated in 1994. Furthermore, these guidelines were revised and published as "Compounding Manuals for Antineoplastic Agents" in 2005. However, Japanese medical professionals were unaware of the risk from exposure to antineoplastic agents while using them. Skin contact with antineoplastic agents while they are being compounded or administered can cause acute dermal reactions such as rashes.[1)] Antineoplastic drugs can have chronic effects on reproduction, causing abortion, stillbirth, infertility, and congenital anomalies.[2–5)] Cancers such as leukemia can also be triggered by such agents.[6)]

The JSHP Academic Committee then drew up the "Guidelines for Compounding Antineoplastic Agents,"[7)] referring to the "ALERT" in "Preventing Occupational Exposure to Antineoplastic and Other Hazardous Drugs in Health Care Settings" announced by the National Institute of Occupational Safety and Health (NIOSH) and the Guidelines from the American Society of Health-System Pharmacists (ASHP). At Yamada Red Cross Hospital (Mie, Japan), we prepared our own manuals for the compounding of antineoplastic agents in refer-

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**Table 1.** Outline of the Revision of the Compounding SOP for Antineoplastic Agents

| Procedures | Previous | Revised |
|---|---|---|
| Wearing of protective gloves when preparing antineoplastic agents for compounding, | none | wear* |
| Wearing of a hair cap while compounding antineoplastic agents | none | wear |
| Changing time of the outer gloves (Wearing of two layers of gloves while compounding antineoplastic agents) | not replaced | replaced for each preparation |
| Shaking of vial to dissolve CP | outside the BSC | inside the BSC |
| Labeling of compounded preparations | outside the BSC | inside the BSC |
| Conveyance of the compounded preparations | on a tray | packaged in zippered vinyl bags |
| Cleaning of floor and tables | once weekly | daily |

*Since it has been reported that the surfaces of product vials are contaminated with antineoplastic agents.[16–18] <Cleaning methods> The floors were cleaned using disposable sheets comprised of polyester, rayon, acrylic, and polypropylene fibers, which were dampened with ethanol and detergent. The tables were cleaned using a disposable nonwoven rayon fabric dampened with ethanol. After completion of compounding, the inside of the BSC was wiped down 4times with gauze, each consecutively moistened with 0.3 M NaOH, 2% sodium hypochlorite, 1% sodium thiosulfate, and finally 80% ethanol in this order.

ence to the above guidelines, and introduced the use of a biological safety cabinet (BSC Class II B) and personal protective equipment (PPE) in the compounding room of the pharmacy department.

In Europe and the U.S.A. it has been reported that medical professionals, who have been cautioned of possible exposure while compounding the agents, have been exposed to antineoplastic agents, detected in urine samples.[8–11] In addition, contamination in medical facilities have been reported in studies testing wipe samples collected in areas used for the compounding and administration of antineoplastic agents. Antineoplastic agents have been detected in many areas such as on surfaces of BSC, floor, storage areas, patient treatment areas, and tables and chairs adjacent to the drug-handling area.[8, 9, 12, 13] In these reports, cyclophosphamide (CP) has been confirmed to vaporize at 23°C,[14] suggesting that pharmacists are not sufficiently protected from exposure, even with the use of safety cabinets. Since these types of studies on the exposure to antineoplastic agents have not been undertaken in Japan, the JSHP Academic Committee selected 6 institutions that handle antineoplastic agents to implement CP detection tests for urine and wipe samples.[15] The Yamada Red Cross hospital participated in this investigation, which indicated that CP was present in urine samples from many medical professionals and at most of the wipe test sites. To improve this situation, the compounding standard operating procedure (SOP) for antineoplastic agents was reviewed by the Department of Pharmacy. The main points of the revision are indicated in Table 1. To validate the revised SOP, CP detection tests for urine and wipe samples were again conducted.

## MATERIALS AND METHODS

**Experimental Schedule** —— Urine samples from 4 pharmacists who compounded CP were collected for 24 hr and measured for CP concentrations in November 2006. In addition, wipe samples were collected at 6 sites in the compounding room. We reviewed the results and revised the compounding SOP. After 5 months, the tests were repeated. The study design was approved by an institutional review board at Yamada Red Cross Hospital and the subjects provided informed consent.

**Urine Tests** —— Four pharmacists (3 males and 1 female) who compounded CP during the period from November 9 to 22, 2006 collected their own urine samples over 24 hr using "Cyto Urine Kits" (Exposure Control B.V., Wijchen, Netherlands) The pharmacists collected the samples at every micturition from prior to compounding antineoplastic agents till the next morning. The samples were collected first in urine receptacles, transferred to vacuum tubes, and then immediately frozen and stored. The collection date, time and volume were recorded. Each pharmacist also recorded the quantity of CP used each time compounding was performed.

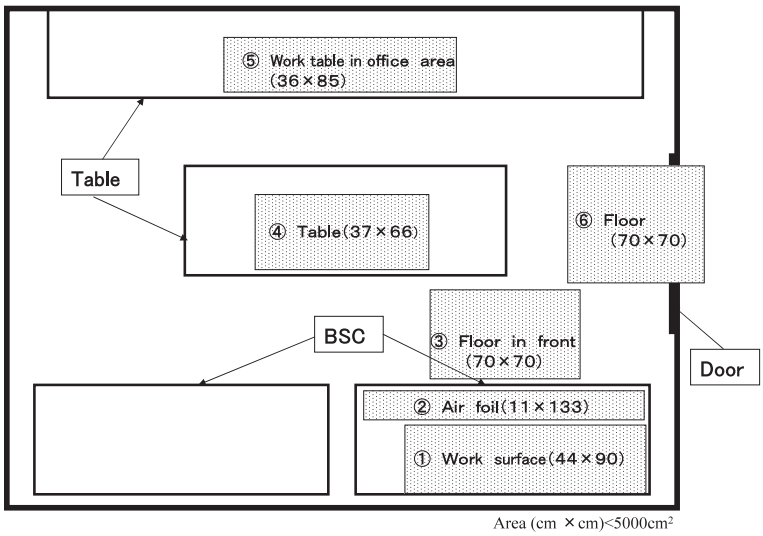



**Fig. 1.** Sites of Wipe Test Sampless in the Chemotherapy Preparation Room of the Pharmacy

In April 2007, a request was made for a revision of the compounding SOP for antineoplastic agents, and after which the 4 pharmacists who participated previously again collected urine samples during the period from August 30 to September 12, 2007.

**Wipe Tests** —— A total of 6 sites in the antineoplastic agent-compounding room of the pharmacy were subjected to wipe tests for CP contamination on November 24, 2006 using Cyto Wipe Kits (Exposure Control B.V.). The test sites were: 1. work surface of the BSC; 2. airfoil inside the BSC; 3. the floor in front of the BSC; 4. the central work table, 5. the work table with a telephone and personal computer in the office area; and 6. the floor of the entry into the compounding room (Fig. 1). The test sites were delineated with masking tape in advance, to calculate the respective areas. To collect test samples, an aliquot of 0.03 M NaOH solution from the Cyto Wipe Kits was applied to each target area, and wiped off twice with dry tissue paper. The tissue paper was then placed in a plastic container with a screw cap, and immediately frozen and stored.

The wipe tests were similarly repeated on September 7, 2007, as were the urine tests.

**Analysis of Samples** —— The analysis of CP levels was contracted out to a Dutch company, Exposure Control, which has a long experience in meeting world standards with cytotoxic drugs. They used gas chromatography-tandem mass spectrometry (GC-MS-MS) for the analysis.

**Statistical Analysis** —— A comparison of the amount of compounded CP and the urinary concentration of CP before and after the SOP revision were made using the Mann-Whitney U test with a significance level of 5%. In addition, the correlation between the amounts of compounded CP and urinary concentration of CP was determined by linear regression analysis.

## RESULTS

### Urine Tests

The mean quantities of CP which the pharmacists compounded on the day of urine tests increased 58% from 2237.5 mg to 3525 mg before and after revision of the compounding SOP, but the change was not significant ($p = 0.06$) (Fig. 2).

CP was detected in urine samples from all 4 pharmacists both before and after the SOP was revised. The urinary concentration of CP decreased for 3 pharmacists and increased for 1 pharmacist after the revision of the compounding SOP. The mean urinary CP before the revision was 165.3 ng/24 hr, and decreased to 47.4 ng/24 hr after the revision, although this difference was not significant ($p = 0.15$) (Fig. 3).

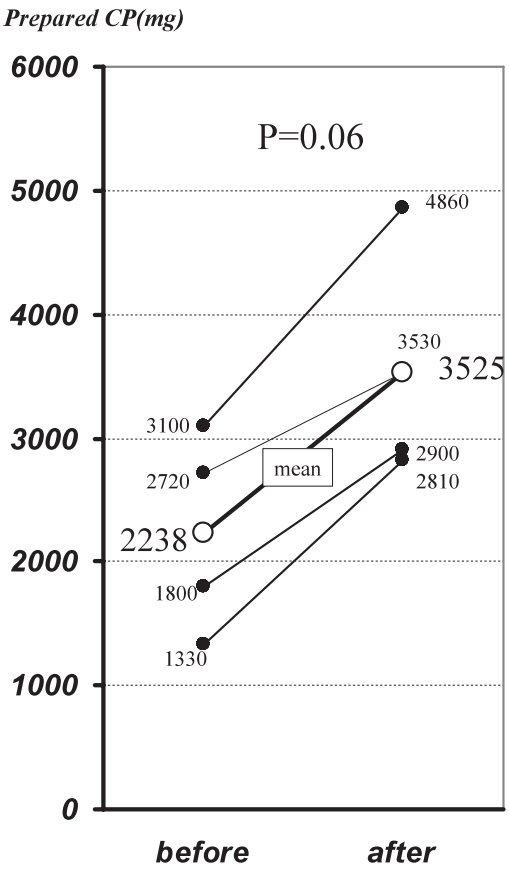
After the compounding SOP was revised, CP was detected in the first urine sample of all pharmacists in the morning even before compounding CP ($3.95 \pm 0.726$).

The amount of CP compounded by each pharmacist before the SOP was not correlated with the urinary concentration of CP detected for the 24 hr, but was significantly correlated with it after the revision of the SOP ($R^2 = 0.87$, $p = 0.033$) (Fig. 4).

**Wipe Tests**

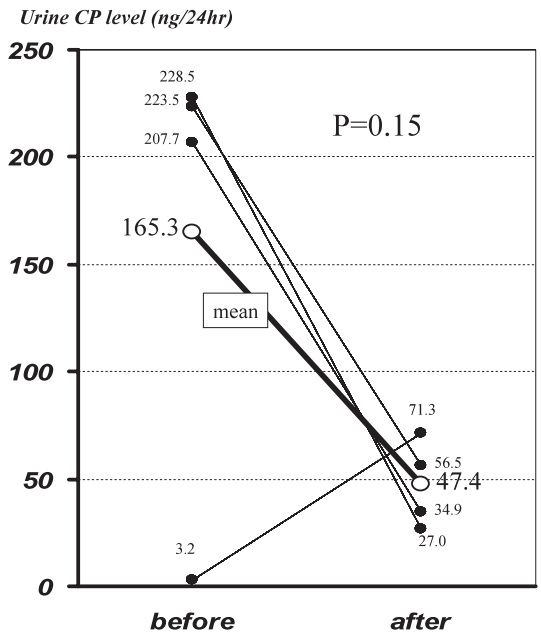
Before the compounding SOP was revised, wipe tests detected CP at all of the test sites in the antineoplastic agent-compounding room of the pharmacy: the work surface and airfoil inside the BSC, the floor in front of the BSC, the central work table, the work table with telephone and personal computer in the office area, and the floor of the entry to the compounding room.

After the SOP was revised, wipe tests detected CP at 4 test sites: the work surface and airfoil inside the BSC, the floor in front of the BSC, and the floor of the entry to the compounding room, but not on the central work table or the work table with telephone and personal computer in the office area. However, more CP was detected in the airfoil inside the BSC than before the SOP was revised (Table 2).




Mean±SE: before 2238±407.3, after 3525±472.9

**Fig. 2.** Amounts of CP Prepared by the Pharmacists before and after Revision of the SOP




Mean±SE: before 165.3±54.2, after 47.4±10.1

**Fig. 3.** Amounts of CP in Urine Samples of the Pharmacists before and after Revision of the SOP

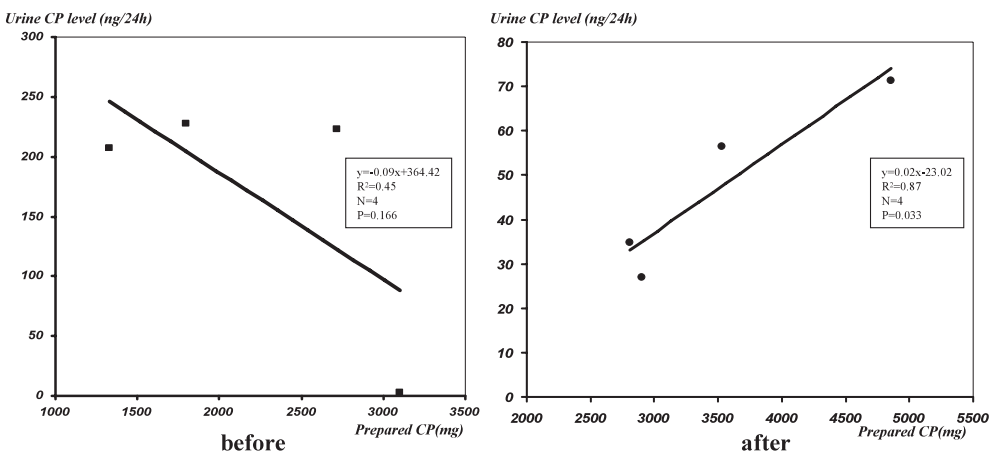



**Fig. 4.** Relationship between the Amounts of CP Prepared and the Amounts in Urine Samples of the Pharmacists before and after Revision of the SOP

**Table 2.** CP in Wipe Samples before and after Revision of the SOP

| Sampling Area | Area Surface ($cm^2$) | Total Volume NaOH(ml) | CP (ng/ml NaOH) | | CP ($ng/cm^2$) | |
|---|---|---|---|---|---|---|
| | | | Before | After | Before | After |
| ①Work surface of BSC | 3960 | 160 | 0.69 | 0.28 | 0.03 | 0.01 |
| ②Airfoil | 1463 | 160 | 0.17 | 0.54 | 0.02 | 0.06 |
| ③Floor in front of BSC | 4900 | 160 | 2.70 | 0.17 | 0.09 | 0.01 |
| ④Table | 2442 | 160 | 0.75 | ND | 0.05 | ND |
| ⑤Work table in office area | 3060 | 160 | 0.22 | ND | 0.01 | ND |
| ⑥Floor of Entry | 4900 | 160 | 0.19 | 0.15 | 0.01 | 0.01 |

ND: Not Detected (CP < 0.10 ng/ml NaOH)

## DISCUSSION

It was surprising for the pharmacists engaged in compounding antineoplastic agents to learn that the first urine tests detected CP in urine samples from all of those tested. Additionally, CP was detected at all of the wipe test sites not only inside of the BSC but also in the compounding room of the pharmacy, confirming that the working environment was contaminated with this antineoplastic agent. A request was then made for a revision of the compounding SOP to prevent further exposure to antineoplastic agents. Urine and wipe tests were repeated 5 months later to confirm the exposure status to antineoplastic agents after the revision of the SOP.

The first tests detected CP at more than 200 ng in urine samples over 24 hr from 3 pharmacists. Sessink *et al.*, have reported an increased cancer risk of 1.4–10 additional cases per year per million workers exposed daily to CP, with an urinary excretion average of 180 ng/24 hr in pharmacy personnel.[19] The mean urinary concentration of CP in the samples from the 4 pharmacists in this study was as high as 165.3 ng close to the 180 ng level reported, but after the SOP was revised this decreased to 47.4 ng. The CP level of 1 pharmacist in the first test was low compared to the other 3 pharmacists. Interviews with the pharmacists revealed that the 3 pharmacists with high CP levels did the shaking step to resolve CP outside of the BSC to hasten the process. This difference in the resolving procedure may be the factor for the CP level difference among the 4 pharmacists' 24 hr urine concentrations. These findings suggest how important it was to revise the method to prevent exposure and making a complete technical standard. In addition, the amount of compounded CP were significantly correlated with the urinary level of CP after the SOP was revised, suggesting that the compounding SOP was standardized and that all pharmacists were exposed through a common mechanism.

After the revision of the SOP, wipe tests in the compounding room of the pharmacy revealed a decrease in CP levels at 4 test sites, the same level at one site, and an increase only in the airfoil of the BSC. Daily cleaning of the surfaces resulted in a decrease in the number of test sites where CP was detected previously, although the cleaning method used inside the BSC appeared to vary. In Japan, NaOH used to clean inside of the BSC has been tested at concentrations of 0.3 and 0.03 M, revealing that the CP detection rate is independent of the concentration of NaOH used, but drops as wiping down is increased.[20] Considering these findings, the inside of the BSC is cleaned by wiping twice with 0.03 M NaOH in our hospital.

Despite the fact that the compounding SOP was improved, CP was still detected in urine samples from all 4 pharmacists and at 4 sites by wipe tests. The direction of air flow in the BSC was suspected to be responsible for this, and was checked using smoke streams from incense sticks placed at several points inside the BSC. However, no smoke leakage could be visually detected. After each compounding for a patient was completed, the pharmacist's outer gloves were, and the removed gloves were then stored in a zippered polyethylene bag inside the BSC. New gloves were worn outside of the BSC. The compounding pharmacists at our hospital did not wear N95 masks but surgical masks, suggesting a possibility that CP which was splashed onto the cuffs of their gowns was inhaled when the gloves were changed. In addition, after the SOP was improved, CP was detectible in morning urine samples collected before the pharmacists even started the compounding operation, suggesting that the pharmacists may have been exposed to antineoplastic agent through the skin because of environmental contamination after completion of the compounding

operation the day before.

Revision of the compounding SOP improved prevention of exposure to CP, but did not lower the level detected in the urine to zero in pharmacists engaged in compounding this agent. Many studies have reported that the PhaSeal® system, a closed medical device to prepare drugs, is useful in decreasing the degree of exposure,[21–25] and this system was introduced to our hospital in April 2008 following careful discussion. However, this medical device is expensive and cannot be introduced to all medical institutions in Japan, given the National Health Insurance (NHI) system. Furthermore, despite advancements in technologies such as electronic medical records in Japan, measures to prevent the exposure of medical professionals to antineoplastic agents are very much underdeveloped. Exposure of medical professionals to antineoplastic agents could be reduced, as in the Netherlands,[19] if urine and wipe tests were conducted at each medical institution by certain organizations or with law, that sets a target for risk levels for exposure. We strongly hope that the JSHP will notify the Ministry of Health, Labor, and Welfare of Japan (MHLW) of the risks in handling antineoplastic agents, and that this will lead to a revision of related institutions or in the NHI system to legally protect medical professionals from exposure to antineoplastic agents, and also that a system will be established for pharmaceutical companies to introduce the use of closed medical devices.

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