Exposure of Pharmacy Technicians to Antineoplastic Agents: Reevaluation after Additional Protective Measures

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ABSTRACT. In the past, special guidelines and protective measures have been introduced to protect hospital workers during the handling of antineoplastic agents; nevertheless, it was found that they did not prevent the uptake of these toxic compounds. In response, additional protective measures were introduced, including adaptations of the laminar downflow hood, use of special masks, use of double pairs of gloves, and replacement of ampules with vials. In the current study, the authors compared the effects in these additional measures with results of a previous study. Cyclophosphamide, 5-fluorouracil, and methotrexate constituted 81% of the antineoplastic agents prepared; therefore, the investigators monitored these compounds again by personal air sampling and by determining the levels of contamination on masks and gloves. Cyclophosphamide in the urine of workers was also measured. During preparation, investigators concluded that there were lower concentrations of cyclophosphamide in the air than had occurred in the previous study. Replacement of ampules with vials (i.e., 5-fluorouracil) resulted in a significantly diminished contamination of latex gloves. Cyclophosphamide was detected in urine samples provided by six of nine technicians; the maximum amount excreted over 5 d was 2.6 μg. The mean cyclophosphamide excretion/d was not significantly lower than that found in the previous study (0.16 μg and 1.44 μg, respectively). Despite an intensified hygienic regimen, exposure to antineoplastic agents cannot be reduced if the reasons for exposure remain unknown.

IN THE NETHERLANDS, special guidelines and protective measures were introduced about 10 y ago to protect hospital workers from health risks associated with handling antineoplastic agents.[1] Recently, however, these measures were amended.[2] Other countries also promote protective guidelines.[3–6] Nevertheless, the results of several studies have shown uptake of antineoplastic agents in hospital workers who have followed the guidelines.[7–15]

In our recent study of the effectiveness of the protective measures, we showed that, during preparation, cyclophosphamide (CP) was detected in the air of the working environment at concentrations of < 0.04–10.1 $\mu g/m^3$.[7] Contamination of, and permeation through, latex gloves were found for the three drugs studied: (1) CP, (2) 5-fluorouracil (5FU), and (3) methotrexate (MTX). In addition, we detected CP in urine samples of some workers. The results showed that even though protective measures were taken, exposure to these compounds occurred nonetheless. More important, the protective measures did not prevent uptake of CP. Investigators suggested that inhalation was of minor importance with respect to internal exposure, compared with other presumably dermal routes.

These results led us to introduce additional protective measures in the hospital. The working aperture of the laminar downflow hood was reduced by enlargement of the viewing screen, and it was decided that the phar-

macy technicians should use double pairs of gloves and a special mask. In addition, technicians used vials, instead of ampules, for 5FU preparation. In the current follow-up study, we investigated the effects of these additional measures. We obtained personal air samples and analyzed them for the presence of CP, 5FU, and MTX. We studied permeation into latex gloves by analyzing the contamination of both the inner and outer gloves, and uptake of CP was determined by analysis of unmetabolized CP in urine.[16] We compared the results of this study with the results of the previous study.

Material and Method

Study design. Nine pharmacy technicians were involved in drug preparation; eight of these technicians were also involved in our previous study,[7] and their codes were the same. They prepared drugs in a laminar downflow hood on 5 successive days (i.e., Monday to Friday). For personal protection, the technicians wore latex gloves, hair nets, and special clothes. In contrast with the previous study, all technicians used a double pair of latex gloves and a special mask. The technicians were aware of the new study design and monitoring process. Any possible effect of this knowledge on their behaviors was not expected because they continued work as usual. The amounts of CP, 5FU, and MTX prepared during this study were 24, 195, and 5 g, respectively (Table 1). These compounds contributed to 81% of the antineoplastic agents prepared. Cyclophosphamide, 5FU, and MTX were packed in vials.

Air sampling. We performed personal air sampling exactly in the manner we had done in our previous study.[7] We took the samples from all nine technicians on 5 successive days.

Masks. We collected the super-sigma 5005-V masks (Filgif, Groeneveld-Dordrecht B.V. [Dordrecht, the Netherlands]) after drug preparation so that no contamination with other materials (e.g., the gloves) was possible. We packed the masks in aluminum foil until time of sample preparation.

Glove contamination. The technicians wore double pairs of Ansell Gammex latex sterile surgical gloves (Ansell Medical [München, Germany) at all times during drug preparation. After they prepared the drugs, we collected the pairs of gloves separately.

Urine sampling. We collected urine aliquots during the 5-d period, starting at the beginning of the time of drug preparation. The urine volumes and excretion periods were registered. We stored the urine samples at –20 °C until the time the samples were prepared.

Sampling procedures, sample preparation, and analysis. Sampling procedures, sample preparation, and analysis are described elsewhere.[8,9,16] The masks were cut into pieces, and we extracted them by sonication during a 90-in period, after which we shook them for 10 min with 250 ml of a 0.03-M sodium hydroxide solution. We then used extracts for direct analysis (i.e., 5FU and MTX) or for further clean-up (i.e., CP).

Statistical analysis. We used SAS software (version 6.06) to conduct the statistical analysis. We quantified correlation with Spearman's rank correlation coefficient; p values refer to two-tailed tests, and only p values ≤.05 were considered significant. In the case of a nondetectable amount, we used half of the detection limit.

Table 1.—Amounts (mg) of Cyclophosphamide (CP), 5-Fluorouracil (5FU), and Methotrexate (MTX) Prepared by Technicians

Technician	Day	CP	5FU	MTX
1	Mon	900	2 600	0
	Tue	650	1 100	0
	Wed	0	38 000	0
	Thu	0	0	75
	Fri	0	1 000	30
2	Mon	1 250	1 100	0
	Tue	1 000	0	0
	Wed	500	0	0
	Thu	0	25 000	0
	Fri	0	0	0
3	Mon	600	4 500	0
	Tue	600	0	0
	Wed	0	1 600	0
	Thu	600	0	0
	Fri	0	25 900	0
5	Mon	1 200	19 900	0
	Tue	0	4 950	30
	Wed	1 150	0	0
	Thu	600	0	0
	Fri	0	900	0
6	Mon	1 000	5 900	0
	Tue	650	0	0
	Wed	550	0	0
	Thu	900	900	0
	Fri	0	17 900	0
7	Mon	0	4 800	0
	Tue	1 200	0	0
	Wed	0	0	0
	Thu	0	0	0
	Fri	0	1 500	15
8	Mon	250	800	0
	Tue	900	1 100	0
	Wed	400	0	0
	Thu	0	0	75
	Fri	0	500	60
9	Mon	650	800	0
	Tue	3 000	900	0
	Wed	0	0	4 910
	Thu	400	25 000	0
	Fri	1 200	900	0
10	Mon	1 000	6 700	0
	Tue	350	0	0
	Wed	1 600	0	0
	Thu	900	900	75
	Fri	300	0	30
Total		24 300	195 150	5 300
Percentage*		9	70	2

*As a percentage of all antineoplastic drugs prepared.

Results

Air samples. We detected no 5FU and MTX on the filters of the personal air samples. We found CP on 2 d; the air concentrations were 0.06 μg/m³ (technician 2 on Wednesday) and 2.0 μg/m³ (technician 8 on Tuesday). On both of these days, technicians had prepared CP. The

limits of detection, depending on the amounts of air sampled, ranged from 0.02 μg/m³ to 0.15 μg/m³ for CP, 1.6 μg/m³ to 9.9 μg/m³ for 5FU, and 3.8 μg/m³ to 24 μg/m³ for MTX.

Masks. Ten of 45 masks were contaminated with CP (Table 2). The amounts of CP ranged from less than 0.2 μg to 8.8 μg. Cyclophosphamide was also present on 2 masks worn by technicians who were not involved in its preparation. We did not find a correlation between level of contamination and the amount of drug prepared or number of vials used. One mask was contaminated with 15 μg 5FU, but no masks were contaminated with MTX. No drugs were detected on the masks worn by five technicians. The limits of detection per mask were 0.2 μg for CP, 5 μg for 5FU, and 13 μg for MTX.

Glove contamination. The outer pairs of latex gloves were most often contaminated with CP (26 pairs) and 5 FU (9 pairs) (Table 3). The amounts of CP and 5FU ranged from less than 0.13 μg to 140 μg (median: 1.2 μg) and from less than 2.0 μg to 450 μg (median: < 2 μg), respectively. One outer pair of gloves was contaminated with 94 μg MTX (i.e., technician 9 on Wednesday). We found 5FU on 1 outer pair of gloves worn by a technician who did not prepare 5FU, and we found CP on 3 pairs of gloves worn by technicians who did not prepare CP. The levels of contamination of the outer pairs of gloves were correlated significantly with the amount of drugs prepared (CP: $r = .60$, $p = .0001$; 5FU: $r = .32$, $p = .03$) and the number of vials used (CP: $r = .63$, $p = .0001$; 5FU: $r = .47$, $p = .001$). We found that 7 inner pairs of latex gloves were contaminated with CP only (Table 3); amounts of CP ranged from less than 0.13 μg to 9.1 μg (median: < 0.13 μg). We found CP on 1 inner pair of gloves worn by a technician who did not prepare CP. The level of contamination of the inner pairs of gloves was not correlated significantly with the amounts of drugs prepared or with the number of vials used. We did not quantify the 5FU contamination of the inner latex gloves because there was interference by coeluting compounds during analysis of the samples. The limits of detection per pair of gloves for CP, 5FU, and MTX were 0.13 μg, 2 μg, and 10 μg, respectively.

CP in urine. We detected CP in urine samples provided by six technicians (Table 4); the maximum amount excreted over 5 d was 2.6 μg. All technicians prepared CP during at least 1 d (mean: 3 d). We found no correlation between the total amount of CP excreted over 5 d and the amount of CP prepared. Even when we averaged the amount of CP prepared by the number of days of preparation, the limit of detection was approximately 0.25 ng/ml urine.

Table 2.—Contamination with Cyclophosphamide (CP) and 5-Fluorouracil (5FU) of Masks Used during Drug Preparation*

Technician	Day	Contamination (μg) CP	5FU
1	Mon	5.5	< 5
	Tue	2.2	< 5
	Wed	< 0.2	15
5	Mon	0.2	< 5
	Thu	0.3	< 5
	Fri	8.8	< 5
8	Mon	2.0	< 5
	Tue	1.8	< 5
	Thu	1.8	< 5
9	Tue	6.2	< 5
	Fri	5.7	< 5

*Only masks with detectable amounts of one of the drugs measured are indicated.

Discussion

In a previous study, we detected CP in personal air samples on 4 of 17 d.[7] The air concentrations ranged from 0.04 μg/m³ to 10.1 μg/m³. In the present study, we once again detected CP, although less frequently (i.e., 2 of 45 d), and the concentrations were significantly lower (Wilcoxon test: $p = .005$). In both studies, we detected no 5FU and no MTX in personal air samples. Adaptations of the laminar downflow hood may have been responsible for the observed decrease in air concentrations of CP. Obviously, the release of aerosols could not be prevented totally, as was demonstrated by the presence of CP on the masks. We did not detect MTX on the masks, but we detected 5FU once—after the preparation of a large amount (i.e., 21 000 mg) of 5 FU.

In both the present and previous studies we observed minimal MTX contamination of the outer latex gloves. In the present study, we found MTX once when a large amount (i.e., 4 910 mg) of MTX was prepared. In our previous study, we found 5FU contamination of the outer latex gloves on 11 of 17 d (range: < 4.0–620 μg), whereas in the present study we observed 5FU contamination on 9 of 45 d (range: < 2.0–450 μg). Both the frequency with which outer gloves were contaminated (Fisher's exact test: $p = .04$) and the level of contamination (Wilcoxon test: $p = .001$) were decreased significantly. Whether the diminished contamination of the outer gloves with 5FU resulted from use of vials—instead of ampules—needs further investigation. Perhaps the breaking of ampules causes a release of aerosols, thus resulting in contamination of the gloves.[7]

During our previous study, we found CP contamination of the outer latex gloves on 8 of 17 d (range: < 0.08–9.6 μg).[7] During the current study, we observed contamination with CP on 26 of 45 d (range: < 0.13–140 μg). We found no significant differences between the frequency of determining contamination of the outer gloves and the level of contamination in the present and previous studies. Breaking of ampules played an important role in our study; this is not surprising because in both studies technicians used vials in the preparation of CP. In our previous study, technicians wore cotton gloves under latex gloves, and we studied the permeation of CP, 5FU, and MTX through the latex gloves. In the present study, we replaced the cotton gloves with latex gloves, which were identical to the

Table 3.—Contamination with Cyclophosphamide (CP) and 5-Fluorouracil (5FU) of Gloves Used during Drug Preparation*

Technician	Day	CP contamination (µg/pair)		5FU contamination (µg/pair)
		Outer gloves	Inner gloves	Outer gloves
1	Mon	4.1	< 0.13	14
	Tue	3.7	< 0.13	< 2
	Wed	< 0.13	2.4	9.1
2	Mon	1.9	< 0.13	< 2
	Tue	< 0.13	< 0.13	26
	Wed	0.37	< 0.13	< 2
3	Mon	54	< 0.13	< 2
	Tue	1.7	< 0.13	< 2
	Thu	2.1	< 0.13	< 2
	Fri	< 0.13	< 0.13	450
5	Mon	83	1.4	12
	Wed	11	< 0.13	< 2
	Thu	5.9	< 0.13	< 2
6	Mon	< 0.13	< 0.13	38
	Tue	0.27	< 0.13	< 2
	Thu	30	< 0.13	< 2
	Fri	2.6	< 0.13	200
7	Mon	< 0.13	< 0.13	37
	Tue	2.9	< 0.13	< 2
8	Mon	2.4	1.3	< 2
	Tue	2.3	< 0.13	< 2
	Wed	1.3	1.5	< 2
	Thu	1.2	< 0.13	< 2
9	Mon	4.5	< 0.13	< 2
	Tue	70	< 0.13	< 2
	Wed	3.4	< 0.13	< 2
	Thu	5.4	3.2	< 2
	Fri	140	9.1	17
10	Mon	0.73	< 0.13	< 2
	Tue	< 0.13	1.3	< 2
	Wed	1.4	< 0.13	< 2
	Fri	1.8	< 0.13	< 2

*Only gloves with detectable amounts of one of the drugs measured are indicated.

Table 4.—Cumulative CP Excretion in Urine of Pharmacy Technicians and Amounts of Cyclophosphamide (CP) Prepared

Technician	CP prepared (mg)	CP in urine (µg/5d)
1	1 550	0.6
2	2 750	ND
3	1 800	ND
5	2 950	2.1
6	3 100	0.2
7	1 200	ND
8	1 550	0.6
9	5 250	1.1
10	4 150	2.6
All technicians	24 300	7.2

Note: ND = not detected.

outer gloves. In the present study, we found that permeation also occurred through the outer latex gloves. In our previous study, 5 of 8 pairs of cotton gloves were contaminated with CP (range: < 0.08–73 µg). Four pairs of these contaminated cotton gloves corresponded to 4 pairs of outer latex gloves, which were not contaminated with CP, therefore suggesting 100% permeation of CP. In the present study, we detected CP on 7 of 45 inner pairs of latex gloves (range: < 2.0–9.1 µg). We found no CP on the corresponding outer pairs of latex gloves from 2 of these contaminated pairs of gloves. As well, we found no correlation between level of contamination on the inner latex gloves and the amount of CP prepared or the number of vials used. However, the sum of the amounts of CP contamination on the outer and inner latex gloves was correlated significantly with the amount of CP prepared ($r = .56$, $p = .0001$) and with the number of vials used ($r = .60$, $p = .0001$). The level of CP contamination on the inner latex gloves was diminished significantly in the present study, compared with the results of the previous study (Wilcoxon test, $p = .02$). Because no difference was observed between both studies with respect to the frequency of contamination of the outer gloves and the level of contamination with CP, we suggest that the diminished contamination was caused by the use of latex gloves as inner gloves. In the previous study, we detected CP in 24-h urine samples provided by five of nine workers.[7] In the present study, we found CP in urine samples provided by six of nine workers, but it should be noted that these

samples were collected over a longer period of time (i.e., 5 d versus 1 or 2 d). We calculated daily mean amounts of CP found in urine and compared the results with the daily mean amounts of CP in urine calculated in our previous study. We found a decrease in mean CP excreted per day—from 1.44 µg in the previous study to 0.16 µg in the current study. The difference, although large, was not significant (paired Wilcoxon test).

In this study, additional protective measures appeared to reduce the external exposure of technicians to CP and 5FU. Methotrexate was barely detected; perhaps this resulted from a higher detection limit of the analytical method. To what extent each of the additional protective measures has contributed to this decrease remains unknown. Nevertheless, contamination of the latex gloves was reduced—possibly from use of vials, instead of ampules. Also, the filters in the personal air samplers contained such small amounts of CP, 5FU, and MTX that we can conclude that these chemicals were barely existent in the air of the working environment. The question remains whether the use of double pairs of latex gloves diminished permeation of CP. Perhaps contamination with CP was decreased because CP permeated the inner pairs of latex gloves. This might have resulted in skin contact and subsequent absorption.

Our aim in this study was to determine whether additional protective measures reduced the uptake of CP. It should be noted that a 90% reduction in exposure levels is important—even if not significant. There might be several reasons for the absence of significance between both studies with respect to the amounts of CP in urine. The absence of significance was likely caused by large standard deviations that resulted from large interindividual differences; we even paired the individual values of the workers between both studies. Absence of significance likely resulted from the small group of workers ($n = 8$). In addition, there is no reason to expect high correlations with external dose when wide coefficients of variation for low exposure levels are observed. Nevertheless, in the current study, the amounts of CP excreted in urine were comparable with the results of urinary CP excretion reported in previously published studies of other groups of hospital pharmacy employees who worked under similar situations and who used comparable protective measures.[9,17]

The question of whether exposure can be diminished by a reduction in handling is a difficult one. Normally, it is reasonable to assume a positive correlation between use and exposure. The question remains as to what extent diminished use will result in a reduction of exposure, because it is not at all clear how exposure occurs. The presence of environmental contamination may result in chronic exposure. We do not expect that diminished use will reduce the environmental contamination present at a given moment in the working area. The results of this study indicate that, despite efforts in a hygienic regimen, exposure to antineoplastic agents cannot be reduced. Only when we know the exact relationship between sources of exposure, distribution, and absorption will we be able to further reduce the level of exposure to antineoplastic agents.

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References

1. Integraal Kankercentrum Noord/Integraal Kankercentrum Oost. Guidelines for Handling and Application of Antineoplastic Agents (in Dutch). Groningen/Nijmegen, The Netherlands: IKN/IKO, 1986.
2. Integrale Kankercentra. Guideline Antineoplastic Agents (in Dutch). Utrecht, The Netherlands: LOK, 1992.
3. American Society of Hospital Pharmacists. ASHP technical assistance bulletin on handling cytotoxic and hazardous drugs. Am J Hosp Pharm 1990; 47:1033–49.
4. Eriksen IL. Handling of cytotoxic drugs: government regulations and practical solutions. Pharm Intern 1982; Aug:264–67.
5. British Pharmaceutical Society. Guidelines for the handling of cytotoxic drugs. Pharm J 1983; 1:230–31.
6. Canadian Society of Hospital Pharmacists. Guidelines for the handling of hazardous pharmaceuticals. Can J Hosp Pharm 1981; July-Aug:37–39.
7. Sessink PJM, van de Kerkhof MCA, Anzion RBM, et al. Environmental contamination and assessment of exposure to antineoplastic agents by determination of cyclophosphamide in urine of exposed pharmacy technicians: is skin absorption an important exposure route? Arch Environ Health 1994; 49:165–69.
8. Sessink PJM, Anzion RBM, van den Broek PHH, et al. Detection of contamination with antineoplastic agents in a hospital pharmacy department. Pharm Weekbl [Sci] 1992; 14:16–22.
9. Sessink PJM, Boer KA, Scheefhals APH, et al. Occupational exposure to antineoplastic agents at several departments in a hospital. Environmental contamination and excretion of cyclophosphamide and ifosfamide in urine of exposed workers. Int Arch Occup Environ Health 1992; 64:105–12.
10. Hirst M, Tse S, Mills DG, et al. Occupational exposure to cyclophosphamide. Lancet 1984; 1:186–88.
11. Evelo CTA, Bos RP, Peters JGP, et al. Urinary cyclophosphamide assay as a method for biological monitoring of occupational exposure to cyclophosphamide. Int Arch Occup Environ Health 1986; 58:151–55.
12. Sessink PJM, de Roos JHC, Pierik FH, et al. Occupational exposure of animal caretakers to cyclophosphamide. J Occup Med 1993; 35:47–52.
13. Sessink PJM, Friemèl NSS, Anzion RBM, et al. Biological and environmental monitoring of occupational exposure of pharmaceutical plant workers to methotrexate. Int Arch Occup Environ Health 1994; 65:401–03.
14. Sessink PJM, Timmermans JL, Anzion RBM, et al. Assessment of occupational exposure of pharmaceutical plant workers to 5-fluorouracil. Determination of α-fluoro-β-alanine in urine. J Occup Med 1994; 36:79–83.
15. Sessink PJM, Cerná M, Rössner P, et al. Urinary cyclophosphamide excretion and chromosomal aberrations in peripheral blood lymphocytes after occupational exposure to antineoplastic agents. Mutat Res 1994; 309;193–99.
16. Sessink PJM, Scholtes MM, Anzion RBM, et al. Determination of cyclophosphamide in urine by gas chromatography-mass spectrometry. J Chromatogr 1993; 616:333–37.
17. Sessink PJM, Kroese ED, van Kranen HJ, et al. Cancer risk assessment for health care workers occupationally exposed to cyclophosphamide. Int Arch Occup Environ Health 1995; 67:317–23.