Monitoring of occupational exposure to antineoplastic drugs

Hedmer, Maria

2006

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MONITORING OF OCCUPATIONAL EXPOSURE TO ANTINEOPLASTIC DRUGS

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Lund 2006
To my family
Monitoring of occupational exposure to antineoplastic drugs

Abstract
Antineoplastic drugs are commonly used in the medical treatment of cancer and some other diseases. Workplaces e.g. hospital pharmacies and wards where antineoplastic drugs are used are contaminated with these drugs and health care workers are therefore at risk of getting occupationally exposed. Many antineoplastic drugs are classified as carcinogenic and teratogenic to humans. Cyclophosphamide (CP) and ifosfamide (IF) are two common antineoplastic drugs. The general aim of this thesis was to develop and evaluate methods for monitoring of exposure to antineoplastic drugs.

Methods for determination of surface contamination and air sampling, both as vapour and particulates, were developed and validated. Furthermore, methods for determination of antineoplastic drugs in urine and plasma were developed and validated. Analysis was performed by liquid chromatography combined with tandem mass spectrometry. The developed methods had high precisions, sensitivities and specificities and were applicable for monitoring of the exposure at workplaces where antineoplastic drugs are used.

To fully validate the biomarker CP in urine, renal clearance of CP was investigated at low plasma and urine concentrations relevant for occupational exposure to CP. This was performed by studying patients treated with CP up to 12 days after the dose. There was no dependence between the plasma concentration and the renal clearance and, thus, CP in urine can be continued to be used as a biomarker of exposure to CP.

External contamination on primary packaging containing CP was evaluated and only low amounts of CP and IF were quantified. Furthermore, surface contamination in a hospital pharmacy and three oncology wards was evaluated. The contaminations were generally low but high amounts were detected on the floors in patient lavatories. No CP or IF was detected in urine from personnel in the studied workplaces, although the cleaners and the assistant nurses did not use adequate personal protective equipment (PPE). The variations of surface contamination seemed to be rather low.

A risk evaluation of occupational exposure to CP in Sweden was performed. Thus, the occupational exposure for cleaners and assistant nurses constitutes a low but not negligible lifetime cancer risk. The risk of reproduction effects cannot be excluded since there is a risk of accidental exposure during pregnancy.

Thus, it is important for these personnel groups to use adequate PPE such as gloves impermeable to antineoplastic drugs and long-sleeved protective gowns. Information and education are also important.

Key words: antineoplastic drugs, cyclophosphamide, ifosfamide, occupational exposure, wipe sampling, air monitoring, biological monitoring, risk evaluation, renal clearance, urine, plasma

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Date

Maria Hedner
April 6, 2006
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LIST OF ORIGINAL PAPERS

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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-OH-CP</td>
<td>4-hydroxycyclophosphamide</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>BSC</td>
<td>Biological safety cabinet</td>
</tr>
<tr>
<td>CAS</td>
<td>Chemical abstracts service</td>
</tr>
<tr>
<td>CL_R</td>
<td>Renal clearance</td>
</tr>
<tr>
<td>CP</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>CP-D6</td>
<td>$^2$H$_6$-labelled cyclophosphamide</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CXCP</td>
<td>Carboxyphosphamide</td>
</tr>
<tr>
<td>CYP</td>
<td>Cytochrome P450</td>
</tr>
<tr>
<td>DCCP</td>
<td>Dechloroethylcyclophosphamide</td>
</tr>
<tr>
<td>5FU</td>
<td>5-fluorouracil</td>
</tr>
<tr>
<td>IARC</td>
<td>International agency for research on cancer</td>
</tr>
<tr>
<td>IF</td>
<td>Ifosfamide</td>
</tr>
<tr>
<td>IS</td>
<td>Internal standard</td>
</tr>
<tr>
<td>i.v.</td>
<td>Intravenous</td>
</tr>
<tr>
<td>i.p.</td>
<td>Intraperitoneal</td>
</tr>
<tr>
<td>LC-MS/MS</td>
<td>Liquid chromatography tandem mass spectrometry</td>
</tr>
<tr>
<td>LD$_{50}$</td>
<td>Lethal dose 50</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantitation</td>
</tr>
<tr>
<td>MTX</td>
<td>Methotrexate</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>NNM</td>
<td>Nornitrogen mustard</td>
</tr>
<tr>
<td>OEL</td>
<td>Occupational exposure limit</td>
</tr>
<tr>
<td>PAM</td>
<td>Phosphamide mustard</td>
</tr>
<tr>
<td>PPE</td>
<td>Personal protective equipment</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>PVDF</td>
<td>Polyvinylidene fluoride</td>
</tr>
<tr>
<td>QC</td>
<td>Quality control</td>
</tr>
<tr>
<td>r</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>t$_R$</td>
<td>Retention time</td>
</tr>
</tbody>
</table>

Yrkesmässig exponering för cytostatika förekommer framför allt vid tillverkning av cytostatika, beredning av infusionslösningar som innehåller cytostatika, behandling av patienter med cytostatika, omvårdnad av behandlade patienter eller städning av lokaler där cytostatika hanteras samt rengöring och service av utrustning. Läkemedlen kan komma in i kroppen via huden eller genom inandning.


Känsliga och specifika metoder för att mäta CP och IF på ytor, i luft samt i urin har utvecklats och testats i denna avhandling. Proverna analyserades med en avancerad utrustning, s.k. vätskekromatografi med kopplad tandemmasspektrometrisk detektion.

För att se om CP i urin går att använda för att mäta exponeringen för CP har en undersökning av njurarnas utsöndringshastighet vid låga plasmakoncentrationer genomförts. Sexton CP behandlade cancerpatienter studerades genom att tre till fyra urin- och blodprover samlades in från varje patient under upptill 12 dygn efter behandlingen. Det visade sig att njurarnas utsöndringshastighet av CP inte var beroende av plasmakoncentrationen, vilket gör det möjligt att fortsätta använda CP i urin för att mäta cytostatikaexponering.

Avstryksprover från läkemedelsförpackningar som innehöll CP analyserades för att utvärdera hur förorenade dessa var. Halterna av CP var låga och bedömdes vara ofarliga för apotekspersonalen som använder personlig skyddsutrustning. På förpackningarna hittades även låga halter av IF, vilket indikerade att detta läkemedel tillverkades i samma fabrik.

Variationen av ytforeningen av CP och IF var ganska låg över tiden, speciellt på de undersökta golven. På de flesta av de undersökta ytorna på de fyra arbetsplatserna fanns låga halter av CP och IF. Dock hittades kraftigt förhöjda halter av CP och IF på golven vid patienttoaletterna på de tre undersökta vårdavdelningarna. Städpersonalen använder inte tillräckligt bra personlig skyddsutrustning och kan därför komma i kontakt med cytostatika via huden. Inga halter av CP kunde påvisas i luften på sjukhusapoteket och inga halter av biomarkörer kunde påvisas i urinen från personalen.

Det kan vara en arbetsmiljörisk att ha långvarig kontakt med dessa läkemedel då skador på arvsmassan kan uppstå. En hög tillfällig exponering kan även ge fosterskador. Det är därför viktigt att man använder rätt personlig skyddsutrustning samt att man hanterar cytostatika så inneslutet som möjligt. Information och utbildning till personal som kommer i kontakt med cytostatika är viktig.
INTRODUCTION

General background
A large number of different drugs are handled daily in the successful medical care of patients around the world. However, in reality all drugs have side effects. Many of the drugs are hazardous and workers who handle them are at risk of getting affected. A group of drugs with high toxicity and carcinogenic properties is the antineoplastic agents used to treat e.g. neoplastic diseases and some non-neoplastic diseases. There are several groups of health care workers including nursing and pharmacy personnel, and cleaners that may be occupationally exposed to these potentially hazardous drugs. Exposure to antineoplastic drugs may be hazardous for workers who handle them. It is therefore important to have proper methods for monitoring of occupational exposure to antineoplastic drugs. The personal exposure can with advantage be assessed by biological monitoring, since there is more than one route of exposure for antineoplastic drugs. However, it is also important to monitor the potential exposure in the workplaces by investigating the degree of contamination.

Antineoplastic drugs
Antineoplastic drugs are a heterogeneous group of agents with antineoplastic properties. The group of agents can be classified into several subgroups depending on their nature or their mechanism of action in the body e.g. alkylating and platinum-containing agents, antimetabolites and antitumour antibiotics (Ringborg et al., 1998). A commonly used group is the alkylating agents including agents such as cyclophosphamide (CP) and ifosfamide (IF). CP has a wide application area and is used in the treatment of e.g. breast cancer, ovarian cancer, lung cancer and different types of leukemia. IF is also widely used in chemotherapy to treat e.g. different malignant lymphoma, testicular cancer and lung cancer. CP is classified as carcinogenic to humans (group 1) and IF is classified as probably carcinogenic to humans (group 2A) by International Agency for Research on Cancer (IARC, 1981 and 1987).
Properties of cyclophosphamide and ifosfamide

CP, 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide, and its isomer IF, 3-(2-chloroethyl)-2-[(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide, are cyclic nitrogen mustard derivates. Both agents consist of a phosphamide ring and two chloroethyl groups. In CP, both chloroethyl groups are attached to the same exocyclic nitrogen, but in IF one of the chloroethyl groups is attached to the endocyclic nitrogen (Figure 1).

![Figure 1. Chemical structures of the oxazaphosphorines CP and IF.](image)

A summary of the chemical and physical properties of CP and IF is shown in Table 1. At room temperature CP is a fine, white, odourless or almost odourless crystalline powder (monohydrate), which liquefies on loss of its water of crystallization. CP is soluble in water and ethanol, slightly soluble in benzene, ethylene glycol, carbon tetrachloride and dioxane; and sparingly soluble in diethyl ether and acetone. CP is sensitive to oxidation, moisture and light. IF also consists of a white crystalline powder at room temperature and it is soluble in water and carbon disulphide. IF is sensitive to hydrolysis, oxidation and heat. Both CP and IF are chiral molecules since they contain a chiral phosphorus atom. The drugs are administered as racemic mixtures of the two enantiomeric forms, (R)- and (S)- (Williams et al., 1999). CP was first synthesised in 1958 by treating N,N-bis(2-chloroethyl)phosphamide dichloride with preparolamine in the presence of dimethylamine and dioxane (Arnold and Bourseaux, 1958). IF was synthesised in 1968 by the cleavage of the aziridine ring of 3-(2-chloroethyl)-2[1-aziridinyl] perhydro-2H-1,3,2-oxazaphosphorine 2-oxide with hydrogen chloride in diethyl ether (Asta-Werke AG, 1968).

Biotransformation of oxazaphosphorines

Oxazaphosphorines act as bifunctional alkylating agents, and thus, they have the ability to form covalent bonds e.g. between two DNA bases. Both CP and
IF are prodrugs and are pharmacologically inactive, which mean that they have themselves no cytotoxic activity. Both agents require biotransformation before their metabolites can cross-link DNA. These cross-links can induce mutations and thereby increase the levels of chromosomal aberrations. There are only minor differences in the metabolism and pharmacokinetics of the R- and S-enantiomer of CP and IF (Jarman et al., 1979).

<table>
<thead>
<tr>
<th>Properties</th>
<th>CP</th>
<th>IF</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAS nr</td>
<td>50-18-0 (anhydrous form)</td>
<td>3778-73-2</td>
</tr>
<tr>
<td>Molecular formula</td>
<td>C$<em>7$H$</em>{15}$Cl$_2$N$_2$P</td>
<td>C$<em>7$H$</em>{15}$Cl$_2$N$_2$P</td>
</tr>
<tr>
<td>Molecular weight (g/mol)</td>
<td>261.1</td>
<td>261.1</td>
</tr>
<tr>
<td>Melting point ($^\circ$C)</td>
<td>41-45 / 49.5-53</td>
<td>48-50</td>
</tr>
<tr>
<td>log $K_{ow}$</td>
<td>0.63</td>
<td>0.86</td>
</tr>
<tr>
<td>Vapor pressure at 20$^\circ$C (Pa)</td>
<td>0.0033$^a$</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$Vapour pressure determined by Kiffmeyer et al. (2002)

Cyclophosphamide
CP is biotransformed in the liver through a group of cytochrome P450 (CYP) enzyme systems e.g. CYP2B6, CYP3A4, CYP2C8, CYP2C9 and CYP2A6, and at least two pathways are involved (Figure 2; Parkinson 2001; Roy et al. 1999; Joqueviel et al., 1998). The major pathway is the formation of 4-hydroxycyclophosphamide (4-OH-CP) by hydroxylation as the initial step of activation of CP. In this pathway 4-OH-CP can be oxidized to 4-ketocyclophosphamide, an inactive metabolite, or exist in equilibrium with the ring-opened tautomer aldophosphamide. Aldophosphamide can either be deactivated by aldehyde dehydrogenase to carboxyphosphamide (CXCP) or spontaneously eliminate acrolein to yield phosphamide mustard (PAM). CXCP is not toxic itself, but may form nonnitrogen mustard (NNM), a potent alkylating agent (Anderson et al., 1995). However, according to Eksborg and Ehrsson (1985) NNM is supposed to have insignificant cytotoxic effect in vivo. PAM is the major cytotoxic metabolite of CP responsible for the antineoplastic activity of CP (Anderson et al., 1995). PAM has a half-life of 40 min in the cell and forms spontaneous the reactive aziridium intermediate, which alkylates DNA. Acrolein is also a cytotoxic metabolite and gives side effects e.g. in the urinary bladder as hemorrhagic cystitis. 4-OH-CP and
aldophosphamide serve as transport forms of PAM and acrolein in the body (Sladek, 1994).

The minor pathway involves the formation of the major inactive metabolite dechloroethylcyclophosphamide (DCCP) and the cytotoxic metabolite chloroacetaldehyde (Joquevial et al., 1998).

**Ifosfamide**

IF is activated via the same pathways as CP. However, during the biotransformation of IF larger fractions of dechloroethylated metabolites and chloroacetaldehyde are formed compared with the biotransformation of CP.

![Figure 2. Metabolic pathways of CP. Steps of enzymatic activation and enzymatic inactivation (-) are shown.](image-url)
Pharmacokinetics of oxazaphosphorines

**Cyclophosphamide**

CP is a small, unionised molecule with a low degree, approximately 20%, of plasma protein binding (Moore et al., 1991). Approximately 13% (range 3-36) of the CP dose is excreted in urine (Sladek, 1994), but there are individual differences in the excretion of CP in urine (Bagley et al., 1973; Milsted and Jarman, 1982; Bailey et al., 1991; Ren et al., 1998; Joqueviel et al., 1998). The mean elimination half-life of CP is approximately 5 hours (range 2-21; Sladek, 1994; Busse et al., 1997), but there are considerable variations in humans (Bagley et al., 1973; de Bruijn et al., 1988; Moore et al., 1988; Busse et al., 1997).

Previously, pharmacokinetics studies performed on patients treated with both conventional and high doses of CP showed that the mean renal clearance ($\text{CLR}_R$) was lower in association with treatment with the conventional doses (Busse et al., 1997; 1999). Therefore, it is unclear if $\text{CLR}_R$ of CP is dependent on the plasma drug concentration of CP. This must be further investigated because if such a dependence exists, biological monitoring of occupationally exposed workers with a biomarker in urine would underestimate the internal dose of CP, and thus, also the risk at the low occupational exposures. Therefore, the biomarker CP in urine needs to be evaluated with regard to $\text{CLR}_R$ of CP at relevant plasma drug concentrations.

**Ifosfamide**

The mean elimination half-life of IF is approximately the same as for CP, 4-6 hours and less than 20% of a dose is eliminated unchanged in urine (Lind and Ardiet, 1993; Boddy and Yule, 2000).

**Toxicity**

Toxic effects of CP have been studied in different animals and its acute toxicity is presented in Table 2 as LD$_{50}$ values (IARC, 1981). From animal studies it has been reported effects on organs *e.g.* bone marrow, lung, gut, pancreas, liver, kidney and urinary bladder (IARC 1981). Also teratogenic effects such as soft tissue and skeletal malformations have been observed in several species such as mice (Porter and Singh 1988), rats (Singh, 1971) and rabbits (Ujhazy et al., 1993). The mutagenicity of CP has been evaluated with Salmonella mutagenicity assays with presence of microsomes and these tests have clearly demonstrated that CP is a mutagen (Benedict et al., 1977; Connor et al., 2000). CP has also been observed to be a carcinogen in several studies (IARC, 1981, 1987) and causes *e.g.* leukemia and tumours in the urinary
bladder of rats (Schmahl and Habs, 1979) and mammary carcinomas and lymphomas in mice (Walker and Anver, 1979). The metabolite chloroacetaldehyde causes nephro- and neurotoxicity (Springate et al., 1997).

**Table 2.** Acute toxicity data of CP and IF presented as LD$_{50}$ values for different animals.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Dose CP (mg/kg)</th>
<th>Dose IF (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats (i.v.)</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>Guinea pigs (i.v.)</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Rabbits (i.v.)</td>
<td>130</td>
<td></td>
</tr>
<tr>
<td>Dogs (i.v.)</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Mice (i.p.)</td>
<td>360</td>
<td>540</td>
</tr>
</tbody>
</table>

The metabolic activation of IF is similar to that of CP, but IF is less toxic (IARC, 1981). However, IF is more nephrotoxic compared to CP due to the fact that more chloroacetaldehyde is formed. A LD$_{50}$ value for IF can be seen in Table 2. Mice treated with IF during pregnancy showed a teratogenic response (Bus et al., 1973). IF has also been reported to be a mutagen (Benedict et al., 1977).

**Health effects in humans**

**Patients**

The therapeutic effect of treating patients with CP and IF is to cause cell death of the tumour cells in the body or to decrease the proliferation of tumour cells. However, normal cells in the body may also be inhibited or killed in association with treatment with antineoplastic drugs. Patients may therefore get adverse health effects such as irritation of the eyes, skin, mucous membranes and respiratory tract. Alopecia, vomiting and diarrhea can occur in connection to chemotherapy treatment with both CP and IF. Also, tissues and organs such as bone marrow, urinary bladder, liver, kidney and heart may be affected by the toxicity of CP and IF (IARC, 1981; Black and Livingstone, 1990). Patients treated with CP for a primary malignancy have an increased risk of developing secondary malignancies e.g. urinary bladder cancer and leukemia (IARC, 1981, 1987; Greene et al., 1986; Travis et al., 1995). Patients treated with CP for non-malignant diseases e.g. rheumatoid arthritis
have an increased risk of developing urinary bladder cancer, leukemia and skin cancer (IARC, 1981, 1987; Baker et al., 1987; Radis et al., 1995).

Four cases of miscarriages due to treatment with CP have been reported (Clowse et al., 2005). Several cases where pregnant women were treated with CP and the embryos thereby were inadvertent exposed in utero and received teratogenic effects have been reported (Greenberg and Taneka, 1964; Toledo et al., 1971; Kirshon et al., 1988; Mutchinick et al., 1992; Zemlickis et al., 1993; Enns et al., 1999; Vaux et al., 2003; Paladini et al., 2004; Paskulin et al., 2005).

Personnel
Health care workers involved in handling of antineoplastic drugs have a potential risk of getting exposed and their health may be affected. Acute health effects such as hair loss, skin rash and light-headedness have been reported from nurses handling antineoplastic drug (Krstev et al., 2003). Valanis et al. (1993a,b) reported a small, but significant increase in the number of acute symptoms from pharmacy personnel and nurses dermally exposed to antineoplastic drugs compared with controls. Also, acute adverse health effects in health care workers have been reported in association with acute events such as accidents (McDiarmid and Egan, 1988). Occupational exposure to antineoplastic drugs may cause liver damages (Sotamiemi et al., 1983) and may have delayed adverse health effects e.g. teratogenicity and carcinogenicity. Since some antineoplastic drugs, e.g. CP, are teratogenic substances, occupational exposure to these drugs may involve a risk of reproduction effects such as infertility, spontaneous abortions and stillbirths (Selevan et al., 1985; Stucker et al., 1990; Valanis et al., 1997, 1999; Dranitsaris et al., 2005; Fransman et al., 2005a). Many antineoplastic drugs, e.g. CP, are carcinogenic to humans and prolonged exposure or high exposure to these drugs can increase the risk of genetic damages, which could initiate tumours. Several studies have reported genotoxic effects such as increased chromosomal aberrations (Burgaz et al., 2002; Cavallo et al., 2005) and increased levels of DNA strand breaks (Fuchs et al., 1995; Undeger et al., 1999) in hospital and pharmacy personnel occupationally exposure to antineoplastic drugs.
Introduction

Monitoring of exposure

Ambient monitoring

Wipe sampling
Wipe sampling is the most common surface sampling method and is used for assessing surface contamination of chemicals (Ness, 1994). Wipe samples provide surface loading data. In earlier studies different wipe sampling methods have been used and CP and IF were detected as surface contaminants in many of the workplaces that were investigated (Sessink et al., 1992a; McDevitt et al., 1993; Minoia et al., 1998; Connor et al., 1999; Schmaus et al., 2002, Larson et al., 2002). However, the validation of these methods has been scarce since different wipe tissues, the use of internal standard (IS) and the stability of wipe samples have only been sporadically evaluated. In addition, the sensitivities of the previous methods have not been high enough to detect and evaluate trace contaminations of CP and IF. Therefore, there is a need to develop and validate a sensitive method to determine trace contamination of CP and IF on surface areas.

Air sampling
For measurements of particulate matter of CP in air different strategies have been used. Stationary samplings were performed as area monitoring by deWerk Neal et al. (1983), Pyy et al. (1988), Sessink et al. (1992a), Sessink et al. (1992b), McDevitt et al. (1993), Sessink et al. (1994a); Kromhout et al. (2000) and as emission monitoring by Pyy et al. (1988), McDevitt et al. (1993) and Minoia et al. (1998). Personal sampling has been performed by Pyy et al. (1988), Sessink et al. (1994a) and Minoia et al. (1998). In these previous methods different types of filters have been used. However, it has been demonstrated that gaseous CP may be present at room temperature (Connor et al., 2000; Kiffmeyer et al., 2002). Therefore, it may not be sufficient to measure only particles of CP in workplace air. In a recent study a stationary sampling method based on filter and cryogenic-trap was developed (Kiffmeyer et al., 2002), but this is unpractical for personal sampling. Another recent method for air sampling of gaseous CP was based on solid sorbent media (Larson et al., 2003), but this method was not validated at realistic air concentrations. Therefore, there is a need to develop and validate sensitive methods for personal sampling of CP in air, both as particles and gas.

Biological monitoring
Occupational exposure to antineoplastic drugs can be assessed by use of a biomarker. A biomarker of exposure should ideally give a measure of the
Introduction

internal dose of the substance that has an adverse effect in the body. Biomarkers should also have long half-lives. As mentioned above, CP is inactive and it is the metabolite, PAM that has an antineoplastic activity in the body. In a study by Joqueviel et al. (1998) urine from patients treated with CP was collected one day after treatment and the fraction of excreted CP dominated the urine (mean 16%), followed by CXCP (mean 10%) and DCCP (mean 3%). Since the mean excretion of PAM and PAM degradation products in urine one day after the dose only was 0.3% it is not possible to measure PAM directly. Several studies have used CP in urine to monitor occupational exposure to CP (Hirst et al., 1984; Evelo et al., 1986; Sessink et al., 1992b; Ensslin et al., 1994a; Minoa et al., 1998; Turci et al., 2002; Pethran et al., 2003; Wick et al., 2003). There are also methods describing the monitoring of IF (Sessink et al., 1992b; Ensslin et al., 1994a; Minoa et al., 1998; Pethran et al., 2003; Wick et al., 2003). However, it would be advantageous to develop more sensitive methods for analysis of CP and IF in urine and plasma.

Occupational exposure

Occupational exposure to antineoplastic drugs may occur in workplaces where antineoplastic drugs are manufactured, prepared or administered to patients. Nursing of treated patients, cleaning and decontamination, waste handling, handling of textiles (e.g. contaminated clothes and beddings) and other activities may also constitute a risk of exposure. Airborne antineoplastic drugs may be present in both particle and gas phase. Dust of antineoplastic drugs may also be present on surfaces. The routes of exposure to antineoplastic drugs are skin absorption, inhalation, ingestion and injection. However, the most likely routes of exposure to antineoplastic drugs are dermal absorption or inhalation of airborne drugs.

In association with manufacturing of antineoplastic drugs dusting may occur in processes such as synthesizing and packaging (e.g. filling of powder into drug vials or tablet production). Wipe sampling was performed in a pharmaceutical plant that manufactured 5-fluorouracil (5FU) and this specific antineoplastic drug was detected on the floor in the drug compounding room at high amounts (Sessink et al., 1994b). Air monitoring has also been performed at pharmaceutical plants in association with drug production of CP (Pyy et al., 1988), 5FU (Sessink et al., 1994b) and methotrexate (MTX; Sessink et al., 1994c) and high amounts of CP in air was detected in connection with manual loading of CP into barrels (810 µg/m³) and during tablet production (360 µg/m³). Biological monitoring of plant workers showed an uptake of 5FU (Sessink et al., 1994b) and MTX (Sessink et al., 1994c), respectively.
Pharmacy and hospital personnel involved in preparation of antineoplastic drugs may be exposed during contact with contaminated surfaces e.g. primary drug packaging (Sessink et al., 1992b; Ros et al., 1997; Delporte et al., 1999; Nygren et al., 2002; Favier et al., 2003; Mason et al., 2003; Connor et al., 2005) and floor and working areas (Sessink et al., 1992a; Sessink et al., 1992b; McDevitt et al., 1993; Minoia et al., 1998; Connor et al., 1999; Kiffmeyer et al., 2002; Schmaus et al., 2002; Wick et al., 2003; Mason et al., 2005). Contact with final drug products (e.g. infusion bags and syringes) might also involve a risk of exposure (Sessink et al., 1992b). Antineoplastic drugs were monitored in the air in pharmacies and hospital wards where antineoplastic drugs were prepared (Sessink et al., 1992a; Sessink et al., 1994a; McDevitt et al., 1993; Minoia et al., 1998; Kiffmeyer et al., 2002; Mason et al., 2005).

Hospital personnel involved in administration of drugs, nursing and care taking of treated patients e.g. handling of patients excreta (urine, vomit, faeces, sweat) or washing patients can be exposed. Surfaces contaminated with antineoplastic drugs have been monitored in hospital wards (McDevitt et al., 1993; Sessink et al., 1992b; Connor et al., 1999; Ziegler et al., 2002; Wick et al., 2003). Antineoplastic drugs have also been found in the air in oncology wards (Kromhout et al., 2000).

Antineoplastic drugs have been found in urine and blood from health care workers (Hirst et al., 1984; Evelo et al., 1986; Sessink et al., 1992b; Ensslin et al., 1994b; Sessink et al., 1994a; Sessink et al., 1994b, Ensslin et al., 1997, Nygren and Lundgren, 1997; Minoia et al., 1998). Although the use of personnel protective equipment (PPE), biological safety cabinets (BSC) and other safety precautions more recent studies have still found antineoplastic drugs e.g. CP in urine from hospital personnel (Turci et al., 2002; Pethran et al., 2003; Wick et al., 2003). It has also been shown that animal caretakers were exposed during work in an animal room where CP treated mice were housed (Sessink et al., 1993).

Since many international studies have monitored antineoplastic drugs in urine from occupationally exposed workers and in workplaces where antineoplastic drug were used and since exposure might involve a potential risk of adverse health effects such as cancer or reproduction effects, there is a need in Sweden to perform a risk evaluation of occupational exposure to antineoplastic drugs.

In general, there is a lack of knowledge of contamination levels on surfaces in Swedish hospitals and pharmacies where antineoplastic drugs are handled. Previously, only one small study has evaluated the surface contamination in a hospital in Sweden and this was in connection with the use of a closed-system for preparation and administration of antineoplastic drugs (Sessink et al., 1999). Furthermore, no information about biological
monitoring of occupational exposed personnel or air monitoring in Sweden exists.

Previous wipe studies investigated only surface contamination of antineoplastic drugs at one sampling occasion and by not knowing how the surface contamination varies over time, there is a problem with the interpretation of the detected surface contamination. Additionally, no studies have evaluated the contamination on primary drug packaging containing blister or on outer packaging of drug vials and how much the handling of these contribute to contaminate surfaces in preparation units.

**Exposure-effects and dose-response relationship**

**Cancer**

There are numerous reports of adverse health effects associated with exposure to antineoplastic drugs. Only a few cancer risk assessments for health care workers occupationally exposed to CP exist although CP has been known to be a carcinogen for many years (Sessink et al., 1995; Sorsa and Anderson, 1996). It is probably the lifetime cumulative uptake of antineoplastic drugs that determines the cancer risk.

The assessment by Sessink et al. (1995) consisted of two parts, one assessment was based on data from animals and one on data from patients. The occupational exposure to CP was based on several studies performed on health care workers (Hirst et al., 1984; Evelo et al., 1986; Sessink et al., 1992a; Sessink et al., 1992b; Sessink et al., 1993; Sessink et al., 1994a; Sessink et al., 1994d) and from those studies the mean daily uptake was estimated to range from 3.6-18 µg (total cumulative uptake of 29-144 mg CP over 40 years).

The animal part was based on data from male and female rats that were daily treated with CP during their lifetime (Schmahl and Habs, 1979). The tumour incidence for urinary bladder cancer and leukemia in rats was 14 and 8%, respectively (Table 3). The risk of health care workers was estimated by linear extrapolation and the lifetime cancer risks of urinary bladder cancer in men and leukemia in both sex were 120-600 cases per million and 95-475 cases per million, respectively.

The second assessment was based on primary tumour data (Baker et al., 1987) and secondary tumour data from patients (Greene et al., 1986). The data is shown in Table 4. The ten-year cumulative uptake of CP during occupational exposure was estimated (7.2-36 mg CP) and linear extrapolation was used to assess the cancer risk.
Table 3. Rats exposed to CP five times a week during life and the risk of tumoursa.

<table>
<thead>
<tr>
<th>Daily CP dose (mg/kg body weight)</th>
<th>Median total CP dose (g/kg body weight)</th>
<th>Urinary bladder cancer risk (per million)</th>
<th>Leukemia cancer risk (per million)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Males</td>
<td>Females</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.31</td>
<td>0.2</td>
<td>59000</td>
<td>0</td>
</tr>
<tr>
<td>0.63b</td>
<td>0.5</td>
<td>56000</td>
<td>0</td>
</tr>
<tr>
<td>1.25c</td>
<td>0.7</td>
<td>140000</td>
<td>0</td>
</tr>
<tr>
<td>2.50</td>
<td>1.3</td>
<td>230000</td>
<td>37000</td>
</tr>
</tbody>
</table>

aFrom Schmal and Habs (1979) and Sessink et al. (1995)
bLowest dose with significant increase in leukemia (Sessink et al., 1995)
cLowest dose with significant increase in urinary bladder cancer in males (Sessink et al., 1995)

The cancer risk of leukemia in women ranged between 17-100 cases per million over ten years (lifetime risk 68-400 cases per million) based on secondary tumours. Based on primary tumours a marginally lower cancer risk of urinary bladder cancer and leukemia was obtained, 15-76 cases per million.


<table>
<thead>
<tr>
<th>Daily CP dose (mg/kg body weight)</th>
<th>Dose CP (g)</th>
<th>Type of tumour</th>
<th>Cancer risk (per million)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8</td>
<td>53a</td>
<td>Urinary bladder cancer and leukemias in both sex</td>
<td>112000</td>
<td>Baker et al. (1987)</td>
</tr>
<tr>
<td>0b</td>
<td></td>
<td>Leukemia in women</td>
<td>1000</td>
<td>Greene et al. (1986)</td>
</tr>
<tr>
<td>0c</td>
<td></td>
<td></td>
<td>54000</td>
<td>al. (1986)</td>
</tr>
<tr>
<td>20d</td>
<td></td>
<td></td>
<td>111000</td>
<td></td>
</tr>
</tbody>
</table>

aMean dose over ten years
bControl group observed during ten years without CP treatment
cMedian dose over ten years
dHighest dose group over ten years
Introduction

(lifetime 60-304 cases per million). However, both the assessments based on either animals and patients seem to agree well.

The assessment made by Sorsa and Anderson (1996) was based on two studies of cancer patients treated with CP (Table 5). In the first study nine out of 602 patients treated with CP for non-Hodgkin’s lymphomas developed leukemia (Pedersen-Bjergaard et al., 1985). In the second study three cases of leukemia was observed among 333 women treated with CP for ovarian cancer (Greene et al., 1986). The incidence of leukemia among the CP treated patients was approximately 1% and the cumulative therapeutic dose of CP was approximated to 4 g. The doses of CP through occupational exposure were estimated not to exceed 0.2 mg per year, which corresponds to a daily dose of 1 µg (200 days a year). The cumulative dose during lifetime occupational exposure (40 years) corresponds to 8 mg CP, which was 0.2% of the therapeutic dose. Sorsa and Anderson estimated the lifetime cancer risk with linear extrapolation to 20 cancer cases per million occupationally exposed.


<table>
<thead>
<tr>
<th>Cumulative CP dose (g)</th>
<th>Cancer risk (per million)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥4a</td>
<td>15000</td>
<td>Pedersen-Bjergaard et al. (1985)³</td>
</tr>
<tr>
<td>≥4a</td>
<td>9000</td>
<td>Greene et al. (1986)⁴</td>
</tr>
</tbody>
</table>

³Assumption of common therapeutic dose (Sorsa and Anderson, 1996)
⁴Data used in cancer risk assessment made by Sorsa and Anderson (1996)

In the current cancer risk assessments for health care workers occupationally exposed to CP, it was assumed that CL_R of CP was independent of the plasma drug concentration of CP. Therefore, there is a need to fully validate the biomarker CP in urine.

Moreover, it is important to remember that the risk assessments were only based on exposure to CP, but in reality, handling of antineoplastic drugs often involves several drugs with similar mechanisms of action, i.e. alkylating properties, and critical effects. Some of these drugs may also be more potent than CP and IF e.g. melphalan (Greene et al., 1986).
Reproduction in humans

Occupational exposure to antineoplastic drugs might also involve a risk of reproduction effects. Treatment with alkylating agents, especially CP, during the first trimester of pregnancy has been associated with the appearance of abnormalities (Greenberg and Tanaka, 1964; Toledo et al., 1971; Kirshon et al., 1988; Mutchinick et al., 1992; Enns et al., 1999; Vaux et al., 2003; Paladini et al., 2004). It has been reported that CP causes teratogenic effects on fetuses such as malformations on the skeleton, face and central nervous system. A summary of doses, gestation time for the exposure and effects can be seen in Table 6.

During the first trimester when the organogenesis takes place the embryo is most vulnerable to exposure to antineoplastic drugs. Clowse et al. (2005) reported of two pregnancies with CP exposure early in the first trimester (gestation week two and three) that both resulted in miscarriages. If the damage to the embryo is severe, spontaneous abortion takes place.

A critical period seems to be between six to eight weeks from conception (Table 6). During the seventh gestational week the segmentation of the limbs and the development of the digits occur (Paladini et al., 2004). Exposure to antineoplastic drugs during this period may result in malformations of the extremities. From patients inadvertent exposed to CP during pregnancy the lowest dose that has been reported to cause teratogenic effect was 200 mg CP (Kirshon et al., 1988).
### Table 6. A summary of cases with in utero exposure to CP and description of effects on the fetuses.

<table>
<thead>
<tr>
<th>Dose CP (mg)</th>
<th>Exposure time (week of gestation)</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 per day(^a) + 1810(^b)</td>
<td>~2 to delivery + ~8</td>
<td>Skeleton and face malformations</td>
<td>Greenberg and Tanaka (1964)</td>
</tr>
<tr>
<td>4×560(^c)</td>
<td>6</td>
<td>Heart malformation, absent toes</td>
<td>Toledo et al. (1971)</td>
</tr>
<tr>
<td>2×200(^d)</td>
<td>2, 7</td>
<td>Face malformations, absent thumbs</td>
<td>Kirshon et al. (1988)</td>
</tr>
<tr>
<td>1200</td>
<td>5-6</td>
<td>Face malformations, absence of fingers and thumb</td>
<td>Mutchinick et al. (1992)</td>
</tr>
<tr>
<td>20 per kg(^e)</td>
<td>6</td>
<td>Skeleton and face malformations</td>
<td>Enns et al. (1999)</td>
</tr>
<tr>
<td>(^f)</td>
<td>0-9 (CP doses twice monthly)</td>
<td>Skeleton malformations</td>
<td>Vaux et al. (2003)</td>
</tr>
<tr>
<td>2×1000(^h)</td>
<td>5, 11</td>
<td>Severe skeleton malformations</td>
<td>Paladini et al. (2004)</td>
</tr>
</tbody>
</table>

*\(^a\)*A daily dose of 100 mg CP was taken from the second week of gestation to the delivery
*\(^b\)*During gestation week eight a dose of 1810 mg CP was administered
*\(^c\)*The patient received four doses of 560 mg CP during gestation week six
*\(^d\)*The patient received a dose of 200 mg CP in gestation week two and seven, respectively
*\(^e\)*The patient received a dose of 20 mg CP/kg
*\(^f\)*No dose information was obtained
*\(^g\)*The patient received a dose of 1000 mg CP in gestation week five and eleven, respectively
AIMS OF THE THESIS

The general aim of this thesis was the development and evaluation of methods for monitoring of exposure to antineoplastic drugs.

The specific aims were:

- To develop and evaluate a sensitive and specific method for wipe sampling of CP and IF on surfaces.
- To develop and evaluate sensitive and specific air monitoring methods for sampling of airborne CP.
- To develop and evaluate sensitive and specific methods for determination of CP and IF in urine and plasma.
- To evaluate the extent of external contamination on primary packaging containing CP.
- To evaluate the levels and the variation of surface contamination of CP and IF in workplaces where antineoplastic drugs are used.
- To investigate CLR of CP at different plasma drug concentrations and thereby validate the biomarker CP in urine.
- To perform biological monitoring on different personnel groups at workplaces where antineoplastic drugs are used.
- To perform a risk evaluation of occupational exposure to CP in Sweden.
MATERIAL AND METHODS

Wipe sampling
Collection of wipe samples was performed with two nonwoven swabs (Hartmann-ScandiCare; Paper I). Each wipe tissue was wetted with 1 ml 0.03 M NaOH solution. Wipe samples collected on floor and working areas were sampled within a plastic frame with an internal size of 20 × 20 cm (400 cm²). Other investigated objects e.g. door handles and primary packaging, had defined surface areas. For each wipe sample a new pair of gloves was used. The investigated surfaces were carefully wiped with a uniform sampling procedure. After the sampling, the wipe tissues were collected in 50 ml polyethylene bottles (Kautex Textron) and stored at 5°C until analysis.

Validation of wipe tissues
A large number of different types of wipe tissues were available. In Paper I, six different types of wipe tissues made of different materials and with different sizes were evaluated (Care Facial tissues, Easi-Tex Master Plus, nonwoven swabs, sterile compresses, Kimcare medical wipes and Swedish filter papers). These wipe tissues were spiked with 5, 10 and 50 ng of CP.

The recoveries of surfaces with different characteristics such as smoothness or roughness were evaluated. In workplaces where antineoplastic drugs are handled e.g. pharmacies and hospital wards surfaces are generally made of laminate, stainless steel or plastic. The absorption capacity of the wipe tissues were investigated by wiping surface areas added with liquid or evaporated spillage of CP and IF. The size of the sampling area was also evaluated by wipe sampling of spiked surfaces areas of either 100 or 400 cm².

A validation was performed between three different persons that wipe sampled spiked evaporated spillage of CP and IF on surfaces made of laminate and plastic (unpublished data).

Application

External contamination on cyclophosphamide packaging
In Paper II, primary packaging of CP (Sendoxan) was investigated. Two packaging of tablets containing 50 mg CP and ten of each drug vial containing 200 mg and 1000 mg CP (in powder form), respectively, were selected. The outside and inside of the outer packaging, blister package, package leaflet,
outside of the drug vials, outside and inside of the vial cap covers and rubber membrane were wipe sampled.

**Surface contamination in workplaces**
Surface contamination of CP and IF were evaluated twice in a hospital pharmacy preparation unit for antineoplastic drugs (Paper II). During the first and second sampling occasion 49 and 19 surface areas, respectively, were wipe sampled. Surfaces such as floor areas in preparation and dressing room, working areas in BSC and different door handles were sampled.

In Paper IV, four different workplaces located at a university hospital were investigated. The investigated workplaces were a hospital pharmacy (previously investigated in Paper II) and three oncology wards, where administration of antineoplastic drugs to patients and nursing and caretaking of treated patients took place. The wards handled different amounts of antineoplastic drugs depending on their specialization of treatment of different cancer diseases. Between 10 and 13 surface areas in each workplace were selected and repeated wipe sampling was performed at randomly selected occasion on the selected surface areas during eight months, approximately once a month. Similar locations were chosen in the three wards to allow comparison.

In connection with the study, an orthopedic ward in the same university hospital was also wipe sampled once (unpublished data). This ward did not handle any antineoplastic drugs. Similar locations were chosen and wiped in this ward as in the oncology wards.

**Air sampling**
For air monitoring of CP, sampling devices consisting of polyvinylidene fluoride membrane filters (Durapore, Millipore) in filter cassettes connected to solid sorbents (Bond Elut, Sorbent) were used (Paper I). The sampling devices were coupled to aspirating low flow pumps (SKC; 0.14 ml/min). The filters and the solid sorbents were stored at 5°C until analysis.

**Validation of filters and adsorbents**

**Filters**
There were many different types of filters commercially available. Five different filters made of four different types of materials, polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), polycarbonate and glass fiber, were evaluated in Paper I. The recovery of the filters was studied by spiking ten filters of each type with 10 ng of CP.
Material and methods

Solid sorbents
In Paper I, two types of solid sorbent were chosen and evaluated (Bond Elut LMS and Abselut NEXUS from Varian). The adsorption and desorption capacity were tested by injecting 200 ng CP into each type of sorbent.

Different parameters such as relative humidity (5 and 60%) and spike amount of CP (5 and 200 ng) were evaluated by using a controlled air system. Air with a constant flow of 200 ml/min was passed through the sorbents during eight hours.

How the recovery was affected by the sampling time was also studied with the controlled air system. Sorbents were spiked with 200 ng CP and air with a relative humidity of 20% was passed through the tubes during one and eight hours, respectively.

To study how different temperatures affected the evaporation of CP, solid sorbents were connected to Teflon tubes that were connected to the controlled air system. A Teflon wool wad containing 200 ng CP was placed into each tube. The evaporation was studied both at room temperature and at 140°C. An air stream with a relative humidity of 20% and a flow of 200 ml/min was passed through the Teflon tubes and into the sorbents during one hour.

Application
In Paper I, stationary air sampling was performed in a pharmacy preparation unit for antineoplastic drugs. During eight hours air was sampled inside a BSC, next to a pharmacy worker (about 50 cm from the breathing zone), above a waste bin and above a shaker.

Biological sampling
Both breast cancer patients and occupationally exposed workers were studied in this thesis. In Paper III, the CLR of CP in 16 female breast cancer patients i.v. treated with conventional doses of CP was studied. Blood and urine were collected at three or four occasions up to 12 days after the chemotherapy treatment with CP.

In connection with wipe sampling of surface areas in the hospital pharmacy and in the oncology wards 22 occupationally exposed workers were biologically monitored by urine sampling (Paper IV).

Collection of biological samples
Blood plasma
Peripheral, venous blood was collected from the patients (Paper III). The blood was collected in 4 ml tubes (Vacuette® K2EDTA, greiner bio-one) and
Material and methods

the blood was centrifuged at 1000 g during 10 min to separate plasma from red blood cells. The plasma was transferred to plastic test tubes with screw cap and stored at -20°C until analysis. A total of 50 plasma samples were collected. At the first sampling occasion an extra tube (3 ml Vacutainer LH PST™ II Plus, BD) of blood was collected for analysis of plasma creatinine, as a measure of renal function, and bilirubin, conjugated bilirubin, alkaline phosphatase, glutamyl transferase, aspartate aminotransferase and alanine aminotransferase, as measures of liver function.

Urine
In Paper III, urine samples were collected from the patients during three or four four-hour periods. Immediately before each period the patient voided and that urine was discarded. All urine produced during the four-hour period was collected in 500 ml polyethylene bottles (Kautex Textron). Patients voided at the end of the collection period and then a blood sample was drawn. The volume of the collected urine was measured and aliquots of 20 ml urine were stored in polyethylene test tubes at -20°C until analysis.

From exposed workers pre- and post shift urine samples were collected in polyethylene bottles (Kautex Textron). The urine was stored in polyethylene test tubes at -20°C until analysis (Paper IV).

In all, 50 and 44 urine samples were collected from patients and workers, respectively.

Analytical methods

Determination of cyclophosphamide and ifosfamide on surfaces
A method for analysis of CP and IF in wipe samples was developed and validated (Papers I-II). In principal, the work-up procedure included addition of IS, 2H6-labelled CP (CP-D6; Phychem), followed by extraction with ethyl acetate and evaporation to dryness under nitrogen gas. The samples were then diluted in 0.5% acetic acid. Standards were prepared by adding 100 µl aliquots from standard working solutions to 1 ml 0.03 M NaOH, followed by the sample preparation procedure.

The wipe samples in Papers I-II, IV were quantified by liquid chromatography tandem mass spectrometry (LC-MS/MS), consisting of a Perkin-Elmer Series 200 LC system with a Series 200 autosampler (Applied Biosystems). A Genesis C18 column (50×2.1 mm; 4 µm; Jones Chromatography) was used. The column outlet was coupled to an API 3000 triple quadrupole mass spectrometer (Applied Biosystems/MDS-SCIEX) equipped with an electrospray ionisation source. The mobile phase consisted of water and methanol containing 0.5% acetic acid. A chromatographic run
took 8.1 min. The flow rate of the mobile phase was 0.2 ml/min and the injection volume 20 µl.

The mass spectrometric analysis was performed using multiple reaction monitoring in the positive ion mode at \( m/z \) 263.1/142.1 (CP analyte fragment), \( m/z \) 261.0/140.3 (CP control fragment), \( m/z \) 261.0/92.2 (IF analyte fragment) and \( m/z \) 261.0/154.1 (IF control fragment) and \( m/z \) 267.1/140.3 (CP-D6).

A standard curve was used to determine the concentrations. Peak-area ratios of the analyte and the IS were used for quantifications. The ratios between the concentrations found by the analyte fragment and the control fragment were not allowed to exceed 20%. If a larger deviation was observed the lowest value was reported, which occurred very rarely.

**Determination of cyclophosphamide in air**

In Paper I, methods for analysis of CP in filter and sorbent samples were developed. The filters were added with 1 ml ethyl acetate and 100 µl IS and then shaken upright for 30 min with an IKA-VIBRA-VXR (IKA Labortechnik). The extracted liquid was transferred to glass test tubes and evaporated to dryness under a stream of nitrogen gas at room temperature. The samples were dissolved in 150 µl 0.5% acetic acid, sonicated for 5 min and then transferred into microvials. Standards were prepared by adding aliquots of 100 µl working solution and 100 µl IS to 1.0 ml ethyl acetate, followed by evaporation according to the sample preparation procedure. The Bond Elut columns were connected to a vacuum system VacMaster 20 (Sorbent) and eluted with 1.5 ml ethyl acetate into test tubes, which then were added with 100 µl IS. The samples were evaporated to dryness under a stream of nitrogen gas at room temperature. Next, the samples were dissolved in 0.5 ml of 0.5% acetic acid, sonicated for 5 min and finally transferred into microvials. Standards were prepared by adding aliquots of 100 µl working solution and 100 µl IS to 1.5 ml ethyl acetate, followed by evaporation according to the sample preparation procedure.

Both filter and sorbent samples were stored at 5°C until analysis. The filter and solid sorbent samples were quantified by LC-MS/MS according the same mass spectrometric settings as for the quantification of wipe samples (Paper I).

**Determination of cyclophosphamide and ifosfamide in biological samples**

Methods for analysis of CP in urine and plasma samples were developed and validated in Paper III and a method for analysis of IF in urine was developed and validated in Paper IV. In principle, the biological samples were added with IS, followed by extraction with ethyl acetate, evaporation to dryness and dissolution in 0.5% acetic acid. The urine and plasma samples were quantified
Material and methods

by LC-MS/MS according the same mass spectrometric settings as for the quantification of wipe samples.

Validation of analytical methods
The developed methods for sampling of CP and IF on surfaces (Papers I-II), CP in air (Paper I), CP and IF in urine (Papers III-IV) and CP in plasma (Paper III) were validated with limit of detection (LOD), within-day and between-day precision and storage stability.

Pharmacokinetic analysis
In Paper III, three or four assessments of CLR were made for each patient. The amount of CP excreted during a sampling interval was calculated as the product of volume and concentration of the urine. Plasma concentrations were analysed at the end of each urine collection interval and the corresponding mid-point concentrations were calculated using the following assumed half-lives: 5 hours up to 60 hours from end of infusion; 10 hours in the interval 60-120 hours from end of infusion; and 42 hours after 120 hours from end of infusion. The CLR during each interval was then calculated as the amount excreted divided by the product of calculated mid-point concentration and the duration of the collection interval. The renal excretion rate of CP was calculated as the amount excreted over the duration of the collection period.

Statistics
In Paper III, mixed model analyses with CLR as dependent variable were carried out using subjects as random effect and different pharmacokinetic variables as covariates. In Paper IV, P-P plots indicated that data were log-normally distributed, and thus all analyses were performed on log-transformed data. Wipe samples taken from the same location but in different wards were considered to belong to the same group. An analysis of variance (ANOVA) considering repeated measures was performed on the log-transformed data and multivariate analysis on interacting terms were performed. The data from the pharmacy was not included in the analysis of the matched surfaces as the surfaces deviated from the oncology wards. To investigate correlations Spearmans rank test was used. Values below the LOD were given the value of half the LOD.

To statistically compare the results from the wipe sampling performed by different persons F-test and t-test were used.

Statistical significance was considered at P values below 0.05.
**Ethics**

The Research Ethics Committee at Lund University (Lund, Sweden) approved the studies of cancer patients (Paper III) and workers (Paper IV). All subjects gave their written informed consent to participate in the studies.
RESULTS WITH COMMENTS

Wipe method

The six different types of spiked tissues had mean recoveries between 88-112% (Paper I). Three types of tissues were excluded (sterile compresses, filter papers and Easi-Tex Master Plus) due to low recoveries and enlarged size. The three remaining wipe tissues had sufficient desorption capacities (Care Facial tissues, Kimcare medical wipes and nonwoven swabs). The nonwoven swabs had the highest absorption capacity of CP, 78-106%, when different surface materials were spiked and then wipe sampled. Thus, nonwoven swabs were chosen.

Wipe sampling of an area of 100 cm$^2$ resulted in mean recoveries between 78-95% while wipe sampling of 400 cm$^2$ resulted in mean recoveries between 84-92%. Wipe sampling on smooth surfaces e.g. made of laminate and stainless steel resulted in slightly higher recoveries for the smaller area (100 cm$^2$) compared with the larger one (400 cm$^2$). Sampling on surfaces with rough characteristic e.g. plastic flooring material resulted in a higher recovery for the larger sampling area than for the smaller. If possible it is preferable to sample a larger area compared to a smaller one, since the likelihood to detect spillage of antineoplastic drugs increases with increasing sampling area. However, it is unpractical to sample too large surface areas. Thus, an area of 400 cm$^2$ was chosen.

Furthermore, it was important to moistening the wipe tissues with a solution that had capacity to effectively clean different types of surfaces. In previous studies a 0.03 M NaOH solution has been used in wipe sampling (Sessink et al., 1992a; Connor et al., 1999). In the present study this solution was also found to give high recoveries and was therefore chosen.

Three different persons performed wipe sampling and the mean recoveries and coefficients of variation (CV) can be seen in Table 7 (unpublished). According to $F$-tests and $t$-tests there were significant differences between person 1 and 2, and 2 and 3 in the result from wipe sampling of CP on laminate. There were also significant differences between person 1 and 2, and person 1 and 3 in connection with wipe sampling IF on laminate. Furthermore, there were significant differences between all three persons in the results from the wiped plastic flooring. However, the differences were small. Two of the persons (No. 1, 2) were trained in wipe sampling while one person was a novice (No. 3).
Results with comments

Table 7. A summary presenting mean recoveries and CVs between three different persons that performed wipe sampling on spiked surface areas made of laminate and plastic. The mean recoveries were based on ten wipe samples.

| Person | Laminate | | Plastic | | |
|---|---|---|---|---|
| | CP | IF | CP | IF |
| Mean recovery (%) | 104 | 3 | 90 | 4 |
| CV (%) | 4 | 95 | 7 | 92 |
| Mean recovery (%) | 97 | 4 | 84 | 4 |
| CV (%) | 4 | 87 | 4 | 86 |
| Mean recovery (%) | 101 | 4 | 86 | 5 |
| CV (%) | 5 | 76 | 10 | 10 |

Validation of analytical methods

To determine the LOD, wipe sample blanks were added with IS and analysed in compliance with the described methods in Paper I-II. The LOD was determined to 0.02 ng/sample for CP and 0.05 ng/sample for IF. The within-day precision for CP and IF was 2% and 4%, respectively. The between-day precision for CP and IF was determined to be 2-5% and 9%, respectively.

Stability tests were made for wipe samples at three different temperatures during two months and a fully description can be seen in Paper I. Wipe samples can be stored at 5 or -20°C for at least two months and for two days at room temperature.

Air methods

Filters

The mean extraction recoveries of the filters ranged between 84-105% (Paper I). Filters made of polycarbonate and glass fiber were excluded due to low mean recovery and high CV and due to break down during the work-up procedure, respectively. The remaining filters, one filter made of PTFE and two filters made of PVDF, seemed to be equally applicable since they had mean recoveries between 97-102% and CVs between 4-7%. However, the Durapore filter with a pore size of 0.65 µm was chosen because it was the cheapest.
**Solid sorbents**

The mean extraction recoveries of Bond Elut and Abselut were 97% and 89%, respectively (Paper I). Thus, Bond Elut was chosen and used further on in this investigation.

Evaluation of different parameters such as relative humidity and spike amount were carried out and the mean recoveries ranged between 86-99%. Solid sorbents spiked with 5 ng CP provided lower mean recoveries and higher CVs than sorbents spiked with 200 ng CP. The relative humidity did not appear to have an effect upon the recovery of CP.

The mean recoveries for the sorbents sampled during one and eight hours were 99% and 97%, respectively. This indicated that it was possible to sample CP for up to eight hours.

Temperature had an effect on the evaporation of CP. At room temperature approximately 11% of the CP was converted into gaseous form during one hour and at 140°C approximately 66% was converted. This demonstrated that CP might be present in the air as a gas at room temperature.

**Validation of analytical methods**

To determine the LODs, blanks of solid sorbent and filter samples were added with IS and analysed in compliance with the methods described in Paper I. The LOD was 0.02 ng CP/sample for the filter method and 0.03 ng CP/sample for the solid sorbent method. The within-day and between-day precisions for the filter method were determined to 4% and 3-8%, respectively. The corresponding values for the solid sorbent method were 5% and 4-6%, respectively. Solid sorbents and filters can be stored for at least 2 months without any loss of CP at room temperature.

**Urine and plasma methods**

The developed methods for determination of CP in urine and plasma are presented in Paper III. The LODs for CP in urine and plasma were determined to be 0.01 and 0.02 ng/ml, respectively. Urine and plasma samples spiked with CP were stored at room temperature during two days and at -20°C for at least one month with no losses.

In Paper IV, the method for determination of IF in urine is described and the LOD was 0.03 ng/ml.

**External contamination on cyclophosphamide packaging**

Contamination on different types of primary drug packaging of drug vials and tablets containing CP were evaluated. CP was detected in 93% of the wipe
samples taken on the drug packaging. IF was also detected on the primary packaging. The complete result is presented in Paper II. The total amount of CP on the primary packaging of the drug vials varied between 6.5-28 ng (Sendoxan 200 mg: median 17 ng) and 10-216 ng (Sendoxan 1000 mg: median 38 ng). The corresponding values for IF were 3.5-85 ng (Sendoxan 200: median 35 ng) and 1.5-4.0 ng (Sendoxan 1000: median 2.4 ng), respectively. The total CP contamination on the two tablet packaging was 8.6 and 15 ng CP, respectively. No amounts of IF above the LOD were found on the tablet packaging.

Investigation of workplaces

Surface contamination

Wipe sampling was performed twice in a hospital preparation unit (Paper II). At the first measurement the median contamination of CP at floors in the preparation room, dressing room and office were 200, 520 and 2.6 pg/cm², respectively. The corresponding median amounts for IF were 7.4, 78 and <0.1 pg/cm², respectively. A high amount of CP, 2100 ng, was found in the refrigerator box used to store CP and IF stock solutions. At the second measurement the median contamination of CP on the floors in the preparation room, dressing room and the room for delivery of drug were 46, 59 and 59 pg/cm², respectively. The corresponding median amounts for IF were 7.2, 1.2 and 110 pg/cm², respectively. The refrigerator box was less contaminated with CP the second time, 20 ng. The results of the other wiped surfaces in the preparation unit are described in Paper II.

In Paper IV, the geometric mean amounts and geometric standard deviations of CP and IF measured on surface areas in the four university hospital workplaces are described. The majority of the sampled floor areas had geometric means within the low concentration range of <90 pg/cm². However, increased amounts of CP and IF on floor areas in patient lavatories in the oncology wards were detected and the amounts of CP and IF ranged between 290-6300 pg CP/cm² and 200-95000 pg IF/cm², respectively. On the floors in patient rooms the highest amount of CP and IF was 140 and 740 pg/cm², respectively. Increased geometric mean amounts of IF were also found on the floor in the utility rooms in ward A (480 pg/cm²) and B (300 pg/cm²). Generally, the geometric mean amounts in the investigated workplaces were lower on working areas compared with floor areas, but the working areas had larger geometric standard deviations (Figure 3).

There were significant differences between the three wards regarding CP contamination (p=0.04) but not with regard to IF (p=0.28). In a corresponding
Figure 3. Geometric mean amounts and geometric standard deviations of (a) CP and (b) IF for floor and working areas in the wards (floor in patient lavatory excluded).
Results with comments

analysis evaluating differences between surface areas regardless of ward both CP (p<0.01) and IF (p=0.02) were found to differ between surface areas. Significant time trends of the geometrical mean surface contamination was seen in ward A (decrease; p=0.02) and ward B (increase; p=0.02) for CP. No time trends were seen for the geometrical means of floor surface contamination in the four workplaces. Furthermore, no correlations between the detected floor contamination of CP and IF and the daily or monthly handled amounts of CP and IF were seen in any investigated surfaces of the workplaces.

In the orthopedic ward neither CP nor IF were detected on any of the sampled surfaces (unpublished).

Air sampling
In Paper I, stationary air sampling was performed in a hospital pharmacy during one workday (=eight hours). A mean air volume of 67 l (range 64-73) was sucked through the air sampling devices. No CP above LOD could be detected in any of the filter or solid sorbent samples.

Renal clearance of cyclophosphamide
In Paper III, blood and urine from cancer patients were sampled up to 285 hours after the CP dose. The plasma concentrations of CP ranged between 0.02 ng/ml and 6.1 µg/ml and urine concentrations from 0.29 ng/ml to 0.14 mg/ml. There was a variation up to nine times in the plasma concentration at the same sampling time between individuals receiving the same doses of CP. CLR and urine flow were between 1.4 to 24 ml/min and 0.13 to 3.5 ml/min, respectively. There was no significant dependence between CLR of CP and plasma concentration of CP (p=0.82). However, there was a significant correlation between CLR of CP and urine flow (p<0.01).

Biological monitoring
CP and IF were not detected in any of the collected pre- and post shift urine samples from the workers at the investigated workplaces in Paper IV.
GENERAL DISCUSSION

Both the use of antineoplastic drugs and the range of applications in medical care have increased over the years (Connor et al., 2005). Also, the doses of antineoplastic drugs have increased. Since antineoplastic drugs must be handled in the medical care, the occupational exposure must be monitored and the risks for the personnel minimized. In order to monitor occupational exposure to antineoplastic drugs in Sweden several monitoring methods were developed and applied in several workplaces where antineoplastic drugs were used.

Wipe method

As reported above several wipe sampling methods for CP exist (Sessink et al., 1992a; McDevitt et al., 1993; Minoia et al., 1998; Connor et al., 1999; Schmaus et al., 2002; Larson et al., 2002). However, the validation of these methods has been scarce. Among the previous methods only Larson et al. (2002) reported that two different types of wipe tissues were evaluated. During the present method development six different types of wipe tissues were evaluated at different concentrations in order to select the wipe tissue with the best properties for wipe sampling. Furthermore, during the work-up procedure IS was added to the wipe samples and this IS consisted of CP-D6, an artificial substance that is not present in hospitals and pharmacies. Most of the previous CP methods used either no IS or IF as IS. Only Schmaus et al. (2002) used CP-D6. The present wipe method was fully validated which included determination of precisions and stability testing of CP during two months at three different temperatures. The stability of wipe samples was also evaluated by Schmaus et al. (2002), but only during four days. The present wipe method was highly specific and sensitive and can therefore detect and evaluate trace contaminations of both CP and IF. Furthermore, wipe samples are simple and fast to perform and inexpensive.

Air methods

Most of the previous air methods sampled only particulate matters of CP (deWerk Neal et al., 1983; Pyy et al., 1988; Sessink et al., 1992a; McDevitt et al., 1993; Minoia et al., 1998). Furthermore, as for the wipe methods, no IS or IF was used as IS. Also, the sensitivity and specificity of the methods were low. During the development of the method it was demonstrated that CP
might be present in gaseous form at room temperature. Connor et al. (2000) and Kiffmeyer et al. (2002) recently demonstrated that the vapour pressure of CP was low but not negligible and, thus, gaseous CP may be present in the workplaces where CP is used. During the evaluation of the present air methods, different types of filters and solid sorbents were used. Furthermore, the samples were added with an artificial IS and the methods were validated with regard to precisions and stabilities of CP. The methods turned out to have both high specificity and sensitivity. With the present air methods it is possible to determine the personal exposure to CP in air, both as particles and gas.

Urine and plasma methods

To monitor occupational exposure to antineoplastic drugs it is important to use urinary methods with high sensitivity and specificity. Furthermore, to be able to measure CL₀₅ at low CP concentrations a highly sensitive plasma method was needed. Thus, highly sensitive analytical methods for determination of CP in plasma and CP and IF in urine were developed. Compared to previous methods for determination of CP in plasma (LOD range 3-10 ng/ml; Sadagopan et al., 2001; Liu et al., 2004) and urine (LOD range 0.04-5 ng/ml; Sottani et al., 1998; Sannolo et al., 1999; Pethran et al., 2003; Kasel et al., 2004) the present methods had lower LODs. Since two fragments, both an analytical and a control fragment, were used for quantification the specificity of the requested substance was very high. Furthermore, the precisions of the methods were good and the stabilities were high. With the methods it is possible to perform biological monitoring of occupationally exposed workers at levels of acceptable risks according to Sessink et al. (1995).

External contamination on cyclophosphamide packaging

Only small amounts of CP were found on the investigated primary packaging. On the majority of the parts of the packaging, small amounts of IF were also quantified. Recently, Mason et al. (2003) also found IF from wipe samples taken on drug vials containing CP. The highest median amounts of CP and IF were found on the outside on the drug vials and vial cap covers. The levels of the CP contamination on drug vials and vial cap covers were lower compared with what Connor et al. (2005) reported (up to 70 µg/vial), but corresponds to what Sessink et al. (1992b), 60 ng/vial, and Favier et al. (2003), 12 ng/vial, found. The detected contamination on CP packaging must originate from the manufacture or packaging process at the pharmaceutical manufacturer, e.g. dusting during the filling process of drug powder into vials or improperly
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washing of the vials. The environment in the drug plants may be contaminated with the drugs produced in the plant. The contamination levels in the present study were low and the results indicate that the cleaning processes used by the manufacturer were effective for preventing contamination with antineoplastic drugs at hazardous levels. However, the results of Connor et al. (2005) indicate that other manufacturers might have problems with contamination of packaging.

Investigation of surface contamination in workplaces

From the exposure measurements in the university hospital, surface loading data was received for workplaces where either preparation of antineoplastic drugs or administration and nursing were performed. The hospital pharmacy was in total sampled nine times and the surface levels of CP and IF were approximately within the same low concentration range. Compared with surface sampling in other pharmacies the present surface loading data on the floor in the drug preparation room was in the same order as reported by Connor et al. (1999; median 110 pg CP/cm², 55 pg IF/cm²), Schmaus et al. (2002; medians 9 and 20 pg CP/cm², 9 and 140 pg IF/cm²), Wick et al. (2003; 9 CP/cm², 1.3 IF/cm²) and Mason et al. (2005; median 29 pg CP/cm², 17 pg IF/cm²), but lower compared to the levels found by Minoia et al. (1998; median 5600 pg CP/cm², 1000 pg IF/cm²). Working areas in the hospital pharmacy were also wiped and the present levels were similar to what Schmaus et al. (2002; medians 17 and 39 pg CP/cm², 9 and 10 pg IF/cm²) and Wick et al. (2003; 5 CP/cm², IF <LOD) found. In general in the present study the contamination levels of CP and IF were low in the hospital pharmacy where antineoplastic drugs were prepared. The surface contamination was probably caused by direct contact with contaminated gloves, clothes or materials or by spillage. As described above, the handling of contaminated primary packaging contributed probably only to a small portion of the detected drug contamination in the investigated preparation unit. Only two different antineoplastic drugs were monitored, but it is realistic to presume that the surfaces in the hospital pharmacy were contaminated with all various antineoplastic drugs that were handled in the workplace.

Connor et al. (1999) wipe sampled floor and working areas in drug administration areas in six different hospitals but no floors in patient lavatories. The loading data on floors (median 150 pg CP/cm², 20 pg IF/cm²) and on working areas (median 50 pg CP/cm², IF <LOD) were in the same range as those in the present study. Also, the surface contamination levels detected by Wick et al. (2003) in drug administration areas were similar to those in the present study. The contamination levels of CP and IF were generally low in the oncology wards. However, in the patient lavatories high
amounts of surface contaminations were found. In the wards, treated patients contributed to contaminate surfaces areas since high concentrations of antineoplastic drugs were excreted in their urine, sweat and faeces. For instance, the high levels of antineoplastic drugs on floors next to patient toilets probably originate from urine spillage during use of the toilet. Since urine from treated patients contains high concentrations of antineoplastic drugs even a very small volume of urine can cause high surface contaminations.

However, the variations in surface contamination on floor areas over time were low. The reason for the low variation in contamination over time is unclear, but it might depend on insufficient removal of CP and IF from surfaces during cleaning. The investigated floor areas were cleaned on a daily basis and the wipe sampling was performed at different times relative to the time of cleaning. Thus, the sampling time did not seem critical. Moreover, no trends over time were seen for any floor contamination in the investigated workplaces and that might indicate that floor contamination does not seem to accumulate over time. Time trends were only seen in ward A and B of the geometrical mean surface contamination of CP. In ward A the surface contamination of CP seemed to decrease over time while the opposite situation prevailed in ward B. Ward A and B handled larger amounts of IF, but no trends over time were seen for IF. Thus, the personnel in the investigated workplaces most likely did not change their behaviour during the measurements.

The variation of contamination on working areas were higher and this might be explained by the cleaning, since the cleaning was performed by the pharmacy and hospital personnel on a more irregular basis compared with the floor areas. Therefore, the cleaning should be further investigated and improvements of the cleaning routines might be needed. However, the daily or monthly handled amounts of CP and IF did not correlate with the measured surface contamination on the floor areas.

In hospitals, antineoplastic drugs are usually used in several wards and treated patients may move between different wards. Therefore, surface contamination of antineoplastic drugs may be distributed to wards that do not use these drugs. It was of interest to examine if surface contamination of CP and IF were present in a ward in the same hospital where no antineoplastic drugs were used. The orthopedic ward may therefore serve as a “control” and possible levels of surface contamination present in this ward can represent the levels of contamination of antineoplastic drugs that may be found in wards not involved in antineoplastic drug handling. However, no amounts of CP or IF were present on the surfaces in the orthopedic ward.
Renal clearance of cyclophosphamide

Patients treated with CP for breast cancer were chosen for the study. Health care workers handling CP are mostly women ranging between 20 and 65 years of age. The design of the study was chosen to mimic the real conditions and the investigated subjects were women 38 to 61 years old.

The patients were treated with CP, epirubicin and 5FU one or several times before they participated in the study and these treatments may have had an effect on their liver and renal function. However, the investigated markers of liver and renal function indicated only a small deviation from the normal range in a few patients and therefore this did probably not affect the results. Moreover, only one patient received a drug known to alter the metabolism of CP and therefore it is not probable that co-administration of other drugs changed the results.

We observed both inter- and intra-individual variations of CL\textsubscript{R} and urine flow. Previously, it has been reported that there are inter-individual and intra-individual variations of the CP pharmacokinetics (Moore \textit{et al.}, 1991; Sladek, 1994; Chen \textit{et al.}, 1995; Busse \textit{et al.}, 1997; Batey \textit{et al.}, 2002) and these variations may be a result of genetic and environmental influences.

CL\textsubscript{R} was evaluated at urine concentrations relevant at occupational exposure to CP. The lowest urine concentration was 0.29 ng/ml and this concentration is in the same range as those CP concentrations, <0.04-2.1 ng/ml, measured in urine from occupationally exposed workers (Minoia \textit{et al.} 1998; Pethran \textit{et al.} 2003; Wick \textit{et al.} 2003).

The finding, that CL\textsubscript{R} was not dependent on the plasma drug concentration of CP, makes it possible to continue to use CP in urine as a biomarker of occupational exposure to CP. However, the biomarker cannot with exactness estimate the internal dose of CP since the dose either might be underestimated or overestimated due to large individual variations in plasma and urine concentrations of CP although the same doses of CP were administered. On the other hand, the biomarker reflects all exposure routes (dermal uptake, inhalation and ingestion) and the use of the biomarker at group level should be more accurate. Furthermore, a dependence between urine flow and CL\textsubscript{R} was discovered. This should be considered when using CP in urine as a biomarker of exposure.

Biological monitoring

By biological monitoring it is possible to get a measure of the actual absorbed dose. A biomarker of exposure should ideally give a measure of the internal dose of the substance that has an effect in the body. In the case of CP it is the aziridinium intermediate of PAM that has antineoplastic activity. In a study by Joqueviel and coworkers (1998) urine from patients treated with CP was
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collected during one day after treatment. One day after treatment the excretion of CP dominated the urine (mean 16%), followed by CXCP (mean 10%) and DCCP (mean 3%). The mean excretion of PAM and PAM degradation products in urine one day after the dose was only 0.3%. It is therefore most suitable to use the biomarker CP in urine to estimate the absorbed and thereby get a measure of the short-term exposure to CP.

An alternative biomarker would be CXCP. One problem with the use of CP as a biomarker is that it is possible to contaminate the urine samples with CP from e.g. the hands but this would not be possible for CXCP.

Twenty-two occupationally exposed workers were biologically monitored. Three of these were pharmacy personnel who prepared antineoplastic drugs and seven were nurses who administered antineoplastic drugs. These ten workers seemed to have adequate PPE in connection with handling of antineoplastic drugs and it was therefore expected not to find any CP or IF in their urine. No CP or IF were detected in urine from the four cleaners and seven assistant nurses, although they used no or insufficient PPE and despite the high levels of contamination of CP and IF in their workplaces. Assistant nurses and cleaners are most likely to come into direct contact with patients excreta during their daily duties and thereby be exposed via the skin. These personnel groups may also come in indirect contact with patients excreta in connection with contact with contaminated surfaces. Research on these personnel groups is sparse although their potential dermal exposure seems not to be neglectable. However, it should be emphasized that only 12 workers from these two personnel groups were monitored. Furthermore, it is likely that the exposure is extensive but is limited to certain days. This accentuates the drawback of a biomarker that only reflects the exposure during one single day.

In recent international studies there are varying results regarding excretion of antineoplastic drug in urine from occupationally exposed workers. For instance, Kiffmeyer et al. (2002) and Mason et al. (2005) found no antineoplastic drugs in urine from workers involved in drug preparation. Furthermore, biological monitoring of different personnel groups in oncology wards was performed by Ziegler et al. (2002) and no CP or MTX were detected in the urine. However, Turci et al. (2002), Pethran et al. (2003) and Wick et al. (2003) found antineoplastic drugs in urine from pharmacy and hospital personnel. The amounts of CP and IF ranged between <0.04->10 ng CP/ml and <0.05-1.9 ng IF/ml, respectively. No cleaners participated in neither of the above mentioned studies.

Risk evaluation

From the evaluation of surface contamination on primary packaging containing CP, low levels of CP and IF were detected. The highest amount of
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CP and IF on a packaging was 216 and 85 ng, respectively. Sessink et al. (1995) estimated the cancer risk to be up to 400 cases per million during a lifetime exposure and with a daily uptake of 3.6-18 µg CP per day. The absorbed doses during handling of these packaging are far from 100%. Thus, the uptake is most likely not harmful regarding cancer risk to the personnel handling and preparing antineoplastic drugs. Furthermore, the personnel who work with antineoplastic drug preparation use extensive PPE including gloves not permeable to antineoplastic drugs. Unprotected handling of the packaging occurs probably only sporadically.

In the four investigated workplaces most of the surfaces were shown to be contaminated with low levels of antineoplastic drugs and most of the personnel groups in these workplaces seemed to use adequate PPE. However, considerable amounts of antineoplastic drugs (up to 95 µg IF/cm²) were detected on floor areas in patient lavatories in the wards. Since the cleaners in the wards had not covered forearms and since they only used thin gloves of vinyl, they were potentially dermally exposed to antineoplastic drugs. A recent Dutch study showed that toilet seats still were contaminated after cleaning with CP amounts up to 4.3 µg/cm² (Fransman et al., 2005b). In that study high amounts of CP were also found on cleaning cloths used for the cleaning of patient toilets. Furthermore, contamination of CP was found on the cleaners hands, foreheads and forearms. Therefore, the toilets themselves are most likely even more contaminated than the floors.

In the present study, knowledge of the surface contamination levels of antineoplastic drugs in a Swedish hospital was obtained. Although more workplaces need to be investigated to gain a more confident assessment of the levels of surface contamination in Sweden, the results give us a hint of the contamination levels. Our surface contamination levels are similar to those reported from hospitals and pharmacies in Germany, Canada, U.K. and USA (Connor et al., 1999; Schmaus et al., 2002; Wick et al., 2003; Mason et al., 2003, 2005). In the study by Wick et al. both wipe sampling on surfaces and biological monitoring of personnel were performed. The surface contamination levels in the Swedish hospital were similar to those of Wick et al. (2003) and Wick et al. detected CP and IF in urine from the workers although they used adequate PPE. Thus, it is by this comparison possible to obtain some hints about the potential risk of working with antineoplastic drugs in Swedish workplaces. In that study the urinary levels were within the same concentration range as those giving a lifetime risk of leukemia and urinary bladder cancer up to 400 cases per million (0.04%) as described by Sessink et al. (1995). Thus, the potential risk for the personnel in the Swedish hospitals cannot be ignored. Moreover, the risk assessment is based on exposure to CP only, but in reality, hospital and pharmacy personnel including cleaners are exposed to a variety of different antineoplastic drugs. IARC has classified ten antineoplastic drugs into group 1 and ten into group 2A (1987, 1990). Many
of these drugs act with similar mechanism e.g. alkylating properties, and give the same critical effects.

Other carcinogenic agents in Sweden e.g. benzene has an occupational exposure limit (OEL) in air that is 1.5 mg/m$^3$ and work at this air concentration during 40 years would lead to a lifetime cancer risk of 600 cases per million (0.06%; Victorin et al., 1993). Moreover, the OEL for benzo[a]pyrene is 2 µg/m$^3$ in air and work at this concentration during 40 years would lead to a lifetime cancer risk of 20000 cases per million (2%). However, the OEL for benzo[a]pyrene was to a great extent influenced by economical and technical considerations (Victorin et al., 1993). The risk level of occupational exposure to antineoplastic drugs assessed by Sessink et al. (1995) is within the same range as the risk level of exposure to benzene.

Furthermore, since some antineoplastic drugs, e.g. CP, are teratogenic, occupational exposure might involve a risk for reproduction effects such as infertility, spontaneous abortions and stillbirths (Valanis et al., 1997, 1999; Dranitsaris et al., 2005; Fransman et al., 2005a). Uptake of antineoplastic drugs during the first trimester of a pregnancy may have an impact on the developing embryo, which undergoes rapid cell division. During the first trimester a woman might not be aware of a pregnancy and an accidental uptake of antineoplastic drugs during this period might affect the embryonic development (Kirshon et al., 1988; Mutchinick et al., 1992; Enns et al., 1999; Vaux et al., 2003; Paladini et al., 2004). Embryos are not protected from conception to the time of discovery of the pregnancy by Swedish legislation (AFS 1994:32, 2005:5) although this time period might be the most important.

Prevention

During handling of antineoplastic drugs it is important to use adequate PPE to prevent occupational exposure to antineoplastic drugs. The practical aspects of the findings in this thesis are that the cleaners PPE need to be improved. Thus, uncovered forearms in combination with use of thin vinyl gloves are not adequate to protect the cleaners against antineoplastic drug exposure. It has previously been demonstrated that thin vinyl gloves are permeable to antineoplastic drugs (Laidlaw et al., 1984) and it is therefore likely that the cleaners hands will be contaminated. Adequate protective clothing during cleaning of e.g. floor and surface areas in patient lavatories is long-sleeved protective gowns and special gloves that are not permeable to antineoplastic drugs. The cleaners must also be informed about the risks of exposure to antineoplastic drugs and be trained to work in environments where antineoplastic drugs are handled. This is also applicable for assistant nurses at risk of getting in contact with urine or other patients excreta.
GENERAL CONCLUSIONS

- A sensitive and specific method to quantify low amounts of CP and IF on surfaces was developed and validated. The method had low LOD, high precisions and sample stability. The method was applicable for monitoring of surface contaminations at workplaces where antineoplastic drugs were used.

- Sensitive and specific methods to monitor CP in air were developed and validated. The methods had low LODs, high precisions and sample stabilities. The methods were applicable for monitoring of air levels at workplaces where antineoplastic drugs were used.

- Sensitive and specific methods for determination of CP and IF in urine and plasma were developed and validated. The methods had low LODs, high precisions and sample stabilities. With the methods it is possible to perform biological monitoring of occupationally exposed workers at levels of acceptable risks.

- The levels of external contaminations of CP and IF on CP packaging were low and assessed most likely not to be harmful to the personnel handling these packaging.

- The detected surface contamination of CP and IF on the majority of the sampled surfaces in the investigated workplaces was low. However, high levels of CP and IF were detected on floor areas in patient lavatories in the oncology wards. The variations in surface contamination levels were rather low.

- CL\textsubscript{R} of CP was investigated at relevant low plasma concentrations and CL\textsubscript{R} was not dependent on the plasma concentration. It is therefore possible to continue to use CP in urine for biological monitoring of CP exposure.

- Biological monitoring was performed on health care workers, but no amounts of neither CP nor IF were detected in any of the urine samples.

- A risk evaluation of occupational exposure to CP in Sweden was performed. Thus, the occupational exposure for cleaners and assistant
nurses involves a low but not neglectable lifetime cancer risk. The risk of reproduction effects cannot be excluded since there is a risk of accidental exposure during pregnancy.
ISSUES FOR FUTURE RESEARCH

Wipe sampling, air monitoring and biological monitoring should be performed in more workplaces where antineoplastic drugs are used.

Cleaners and assistant nurses potential and actual dermal exposure to antineoplastic drugs should be further investigated by different sampling techniques such as patch, washing, wiping or tape-stripping.

The efficiency of removal of the antineoplastic drugs during cleaning should be further studied.

Reproduction toxic effects among health care workers exposed to antineoplastic drugs should be investigated.

CXCP should be investigated as a biomarker of exposure to CP.

To evaluate if CP and IF are present at primary packaging of other drugs.
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REFERENCES


References


References


References


Ness SA. Surface and dermal monitoring for toxic exposures. 1994, Van Nostrand Reinhold, New York, USA.


References


References


References
