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Leukemia from Dermal Exposure to Cyclophosphamide among Nurses in the Netherlands: Quantitative Assessment of the Risk

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ABSTRACT

Several studies showed that oncology nurses are exposed to antineoplastic drugs via the skin during daily activities. Several antineoplastic drugs (including cyclophosphamide) have been classified as carcinogenic to humans. This study aims to assess the leukemia risk of occupational exposure to cyclophosphamide. Average task frequencies from the population of oncology nurses in the Netherlands and task-based dermal exposure intensities were used to calculate oncology nurses' dermal exposure levels. A dermal absorption model in combination with a physiologically based pharmacokinetic model was used to assess the delivered dose of cyclophosphamide and its active metabolites in the bone marrow. This delivered dose was subsequently related to pharmacodynamic and epidemiological information from a longitudinal study with cyclophosphamide-treated patients to estimate the excess lifetime leukemia risk at age 80 for Dutch oncology nurses after 40 years of exposure to cyclophosphamide. The excess lifetime leukemia risk at age 80 of an exposed oncology nurse after 40 years of dermal exposure to cyclophosphamide was estimated to be 1.04 per million oncology nurses. This risk could potentially increase to a maximum of 154 per million if a nurse performs all cyclophosphamide-related tasks with the maximum frequency (as observed in this population) and is exposed to maximum exposure intensities for each task without using protective gloves for 40 years. This study indicates that the risk of an oncology nurse in a Dutch hospital with an average dermal exposure to cyclophosphamide is well below the maximum tolerable risk of one extra death from cancer per 250 deaths after 40 years of occupational exposure, and that this level is not exceeded in a worst-case scenario.

KEYWORDS: antineoplastic drugs; cyclophosphamide; leukemia; nurses; occupational dermal exposure; pharmacokinetic model

INTRODUCTION

Exposure to antineoplastic drugs among pharmacy technicians and nurses has been extensively studied during the last two decades. Numerous studies have shown that nurses (and other hospital workers) are exposed to antineoplastic drugs during daily activities, and antineoplastic drugs have been detected in oncology nurses' urine (Burgaz *et al.*, 1999; Ensslin *et al.*, 1994; Sessink *et al.*, 1994). Some studies evaluated exposure by inhalation and found very low levels of these drugs in workroom air (deWerk Neal *et al.*, 1983; Kiffmeyer *et al.*, 2002; Kromhout *et al.*, 2000; McDevitt *et al.*, 1993). Other studies have pointed out that exposure via the skin is the major route of exposure (Fransman *et al.*, 2004, 2005; Kromhout *et al.*, 2000). Although exposure by inhalation cannot be entirely ignored, it seems to be negligible in comparison with the dermal route of exposure. In contrast to studies on exposure to antineoplastic drugs, only few studies focused on adverse health effects of these occupational exposure levels. Because of the very low expected incidence of cancer caused by exposure to antineoplastic drugs, it will be very difficult to directly measure this risk in human subjects. No epidemiological studies on the relation between cancer and exposure to these drugs have been performed, and a modeling simulation therefore seems the most appropriate way to provide evidence of the likely magnitude of risk. On the other hand, reproductive health effects of these exposures have been investigated (Fransman *et al.*, 2007b; Selevan *et al.*, 1985; Stücker *et al.*, 1990, 1993; Valanis *et al.*, 1999, Lawson *et al.*, 2012). Based on reported side effects among patients as well as animal experiments, several antineoplastic drugs, up till now, have been classified by the International Agency for Research on Cancer (IARC) to be carcinogenic to humans (group 1) (www.iarc.fr), including cyclophosphamide (CP), which is one of the most frequently used anticancer drugs. CP, when administered as chemotherapy to patients at therapeutic doses (150 mg–6 g per administration), is known to cause secondary malignancies in patients treated with this drug (Baker *et al.*, 1987; Curtis *et al.*, 1992; Greene *et al.*, 1986; Haas *et al.*, 1987; Kaldor *et al.*, 1990). The cancer risk associated with occupational exposure has been investigated previously (Sessink *et al.*, 1995), but the uptake of CP in that study could only be based on urinary excretion of CP in healthcare workers and did not estimate the relevant dose of active metabolites (responsible for the cytotoxicity of CP) in target organs. A more recent study provided dermal exposure levels during daily nursing activities (Fransman *et al.*, 2005), which enabled the assessment of dermal uptake and subsequent distribution to target organs of CP and active metabolites.

This study aimed at assessing the leukemia risk of occupational exposure to CP by assessing the delivered dose of CP and its metabolites in target organs and to combine these with pharmacodynamic information from studies with CP-treated patients. Because CP is a pharmaceutical, the kinetics and side effects have been well investigated and described, which in combination with the available quantitative task-based dermal exposure measurement data makes this study unique in this type of occupational risk assessment studies.

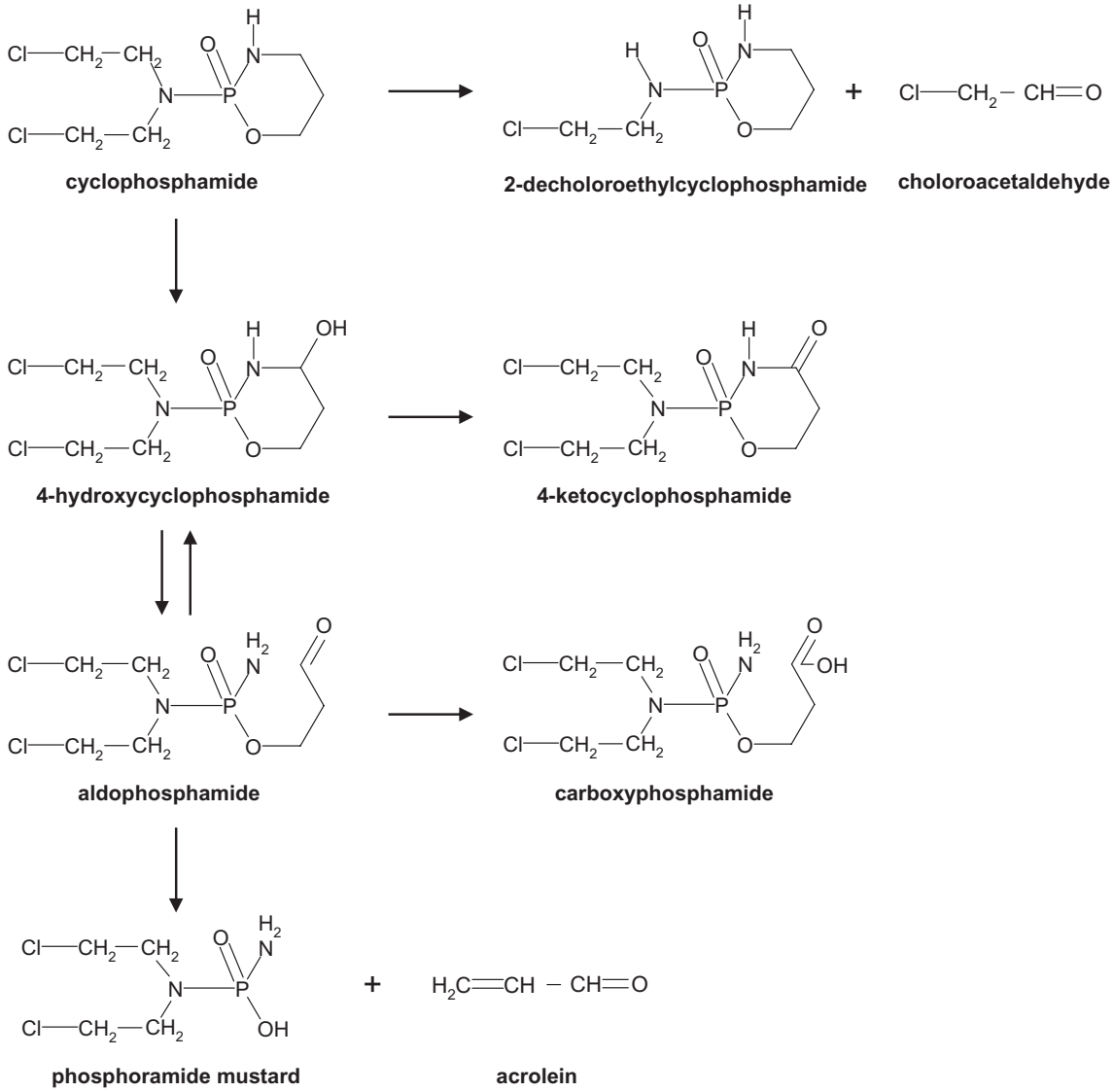
MATERIALS AND METHODS

CP metabolism

CP is one of the most widely administered anticancer drugs and is also used for its immunosuppressive action. CP is an inactive prodrug and is activated in the liver to form 4-hydroxycyclophosphamide (4OH-CP) (Connors *et al.*, 1974; Grochow and Colvin, 1979; Sladek, 1988) (Fig. 1). 4OH-CP readily diffuses into cells (Boyd *et al.*, 1986) and spontaneously decomposes into phosphoramide mustard and acrolein (Connors *et al.*, 1974), which are considered to be the major cytotoxic metabolites and presumably responsible for the carcinogenic potential of CP (Anderson *et al.*, 1995; Boyd *et al.*, 1986). In competition with the activation pathway, both CP and 4OH-CP are irreversibly deactivated to 2-decholoroethylcyclophosphamide, 4-ketocyclophosphamide, and carboxyphosphamide (Busse *et al.*, 1999; Joqueviel *et al.*, 1998; Ren *et al.*, 1998; Tasso *et al.*, 1992) (Fig. 1).

Estimation of dermal uptake of CP

Although exposure by inhalation cannot be entirely ignored, exposure to CP through the skin is considered to be the major route of exposure during nurses' daily activities, and the internal dose of CP was estimated based on dermal absorption. Dermal absorption of CP was assessed by using the methodology described by Cleek and Bunge (1993). The main assumption in this



1 Metabolism of CP. Inactivation pathways are illustrated horizontally, and activation pathways are shown vertically.

approach is that permeation through the skin is well described by the diffusion equation:

$$\frac{d[\mathrm{CP}]}{dt} = D_{\mathrm{SC}} \frac{d^2[\mathrm{CP}]}{dx^2}$$

which relates the change in [CP] to the diffusion coefficient of the stratum corneum (D_{SC}) and position x in the stratum corneum D_{SC} is correlated to molecular weight (MW) and octanol/water partition coefficient ($\log(K_{ow})$) via QSARs (Cleek and Bunge, 1993). The thickness of the stratum corneum (L_{SC}) was estimated to be 18 μm for the hands (Cleek and Bunge, 1993). The spatial variable x represents the continuous position in the stratum corneum. To solve the continuous diffusion equation, we divided the thickness of the skin (L_{SC}) into 100 compartments, which resulted in a system of 100 coupled linear differential equations. These equations were incorporated in the physiologically based pharmacokinetic (PBPK) model and were integrated using numerical software. We validated the implementation by comparing steady-state scenarios

with analytic solutions of the diffusion equation (Cleek and Bunge, 1993).

Frequencies of task performance with antineoplastic drugs derived from a recent questionnaire survey among the entire population of Dutch oncology nurses (Meijster *et al.*, 2006) were used to hypothesize an oncology nurse's average long-term exposure (Table 1). The assumption was made that CP covers approximately 25% of all administered antineoplastic drugs. Average time intervals between tasks were calculated to be used as points in time during an oncology nurses' 40-year working period at which dermal exposure 'pulses' were inserted into the PBPK model. According to Dutch regulations, nurses have to use gloves during all tasks with antineoplastic drugs (or excreta from patients treated with antineoplastic drugs). The dermal exposure model assumed that hands were washed (with assumed 100% removal of contaminant) by oncology nurses directly after the end of the task and after removal of gloves. Therefore, arithmetic mean dermal exposure levels measured on the skin of Dutch nurses' hands underneath gloves for each task (except for the task 'administration of CP') (Fransman *et al.*, 2005) were used as input in the PBPK model. For the task 'administration of CP', dermal exposure measurement data were not available, and therefore, data were imputed using data from surface wipe samples of IV infusion bags (Fransman *et al.*, 2005), with the worst-case assumption that 100% transfer occurred from the infusion bags onto the gloves and with the same 93% protection of gloves as during the preparation of CP (Fransman *et al.*, 2005) (Table 1). In The Netherlands, preparation of CP is not performed by nurses but exclusively done in the hospital pharmacy by trained pharmacy technicians using a strict control regime. Therefore, the preparation and handling of (potentially contaminated) drug vials were not considered in this study, which is primarily focused on exposure of nurses.

In addition, a worst-case dermal exposure scenario was used as input for the PBPK model by using the maximum task frequency for each of the tasks with exposure to CP (Meijster *et al.*, 2006) and the maximum intensity of exposure to CP when no gloves were used during the tasks (Fransman *et al.*, 2005) (Table 1).

PBPK modeling

Of all cancers, non-lymphocytic leukemia has been most frequently found after CP treatment, not only as secondary malignancy but also as primary malignancy after CP treatment for non-neoplastic diseases (Baker

Table 1. External dermal exposure input for the PBPK model

Task	Task frequency for antineoplastic drugs[a] (maximum)	Occurrence of task with CP[b] (maximum)	Arithmetic mean dermal CP exposure levels on hands[c] (maximum)	Estimated dermal exposure duration
Administering	3.62× per week (80× per week)	Once every 8 days (once every 8 h)	1.13 ng (16.2 ng)	10 min
Handling urine	5.24× per week (60× per week)	Once every 5 days (once every 11 h)	28.56 ng (202.6 ng)	5 min
Washing patient	1.88× per week (18× per week)	Once every 15 days (once every 37 h)	34.50 ng (788.1 ng)	20 min
Changing bed sheets	3.13× per week (60× per week)	Once every 9 days (once every 11 h)	40.00 ng (230.0 ng)	10 min
Cleaning activities	2.57× per week (14× per week)	Once every 11 days (once every 48 h)	12.50 ng (800.0 ng)	20 min

[a]Results derived from a recent questionnaire survey among oncology nurses in the Netherlands (Meijster *et al.*, 2006).
[b]Assuming that CP covers approximately 25% of all administrations with antineoplastic drugs.
[c]Results derived from a dermal exposure measurement study (Fransman *et al.*, 2005) by only using measurement data from nurses who used gloves.

et al., 1987; Greene *et al.*, 1986; Haas *et al.*, 1987; Kaldor *et al.*, 1990). Therefore, the bone marrow was considered to be the main target site for this toxicity parameter of the active metabolites of CP (phosphoramide mustard and acrolein), and the delivered dose of 4OH-CP to this organ was simulated. Both the parent compound and main metabolites present in the blood compartment (4OH-CP, aldophosphamide, 2-decholorethylcyclophosphamide, 4-ketocyclophosphamide, and carboxyphosphamide) were simulated in the PBPK model. Pharmacokinetic data were calculated using SCoP (http://www.simresinc.com/). Physiological parameter values for the PBPK models were derived from Brown *et al.* (1997) (Table 2). The octanol/water partition coefficient for 4OH-CP was extrapolated from the octanol/water partition coefficient of CP by using the software package and chemicals database EPI-suite made available by the Environmental Protection Agency (http//www.epa.gov/oppt/exposure/docs/episuitedl.htm). Tissue/blood partition coefficients were calculated from the octanol/water partition coefficients of CP and 4OH-CP using the model by Balaz and Lukacova (1999). The tissue/blood partition coefficients for bone marrow and skin were calculated from Poulin and Theil (2000) (Table 2).

The delivered dose to bone marrow in CP-treated patients was similarly estimated as for the oncology nurse, using the patient's administered intravenous dose inserted into the blood compartment of the PBPK model.

Leukemia risk

Several epidemiological studies have reported secondary malignancies or primary malignancies after treatment with CP (Baker *et al.*, 1987; Greene *et al.*,

Table 2. Physiological parameter values and tissue–blood partition coefficients for CP and 4OH-CP (based on Balaz and Lukacova, 1999; Brown *et al.*, 1997; Poulin and Theil, 2000)

Tissue	% Body weight	% Blood flow	Tissue–blood partition coefficients CP	Tissue–blood partition coefficients 4OH-CP
Kidney	0.4	8.75	1.05	0.97
Liver	2.6	11.4	1.05	0.91
Exposed skin			1.13	
Bone marrow	0.04	0.04		0.77
Richly perfused	5.1	57.7	1.06	0.98
Lung	2.6	50	0.99	0.97
Heart	0.5	2.0	1.06	0.92
Brain	2.0	5.7	1.16	1.00
Slowly perfused[a]	82.9, 78.7	22.2, 20.2	1.44, 0.5	0.73, 0.5
Skin	3.7	2.9	1.13	0.87
Fat	21.4	2.6	2.23	0.26
Muscle	40.0	9.5	1.03	0.97
Bone	10.1	0.1	1.50	0.77
Other	3.5	5.1	0.5	0.5
Blood	7.9			

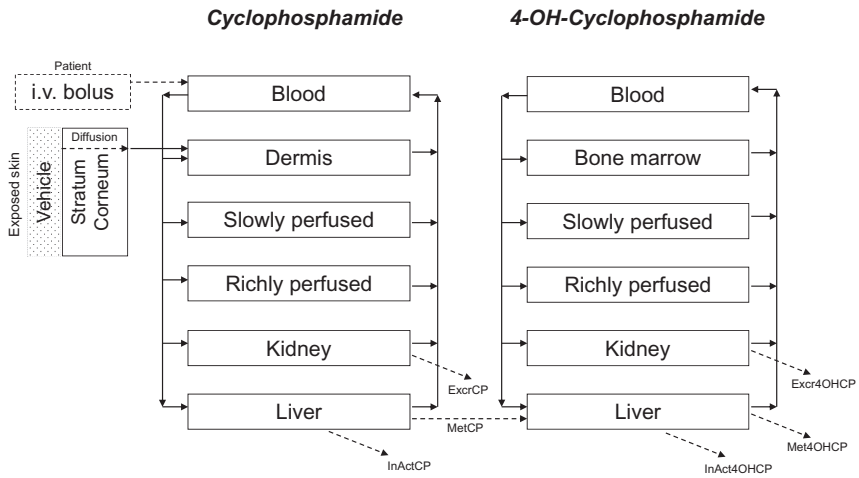
[a]The composition of the slowly perfused compartment differs for CP and 4OH-CP. The first value in the cell describes CP variables, and the second value describes 4OH-CP variables.

1986; Haas *et al.*, 1987; Kaldor *et al.*, 1990). Non-lymphocytic leukemias were most frequently found. In one of these published studies (Greene *et al.*, 1986), the administered CP dose, duration of CP treatment, and cumulative cancer incidence after a complete follow-up of 10 years were reported, and these data were therefore considered suitable to be used in this risk assessment. The cohort study by Greene *et al.* included 3363 1-year survivors of ovarian cancer who were treated in two large medical centers, of which 111 patients were treated with a high dose of CP. The median monthly administered dose for these patients (3.86 g) was inserted into the same PBPK model (Fig. 2) using the same physiological parameters and partition coefficients, but now directly into the blood compartment (intravenous administration). Since data from an animal study (Schmähl and Habs, 1979) showed a linear dose-response curve between daily CP dose and cancer risk, linear extrapolation from high- to low-delivered dose in bone marrow was used to estimate the cumulative leukemia risk for an oncology nurse occupationally exposed to CP on the skin for 40 years.

Excess lifetime risk calculations

Excess lifetime risk from exposure to CP was calculated using life table techniques accounting for all-cause mortality, applying an adaptation of the method described in the BEIR report (Ellett, 1988). Excess lifetime risk was calculated through age 80 for an average exposed oncology nurse and for the worst-case scenario.

Background mortality rates were obtained from Dutch vital statistics (Statistics Netherlands) for the years 2001–2010 stratified by 5-year age groups, and the probability of surviving each age interval was calculated. Dutch leukemia incidence rates (Netherlands Cancer Registry) were obtained for the same time period and similarly stratified by 5-year age groups. Subtypes included in the definition of leukemia were 'Precursor-cell lymphoblastic leukemia/lymphoma', 'Acute Myeloid Leukemia', 'Other myeloproliferative neoplasms', and 'Non-Hodgkin Lymphoma, indolent and chronic lymphatic leukemia'. For each age interval, the cumulative probability of incident leukemia was calculated, conditional on not dying from other causes. Age-specific probabilities of contracting leukemia were then summed across age groups to get the background (unexposed) lifetime (up to age 80 years) risk of incident leukemia. This same procedure was then repeated for the exposed group, except that the age-specific background leukemia incidence rates were multiplied by the rate ratio predicted by the exposure-response model for the cumulative exposure for that age group. Age-specific cumulative exposures were derived by assuming a constant exposure intensity. Excess lifetime risk was derived by subtracting the cumulative risk in the non-exposed population from the cumulative risk in the exposed population.



2 Compartmental flow models for CP and 4OH-CP.

RESULTS

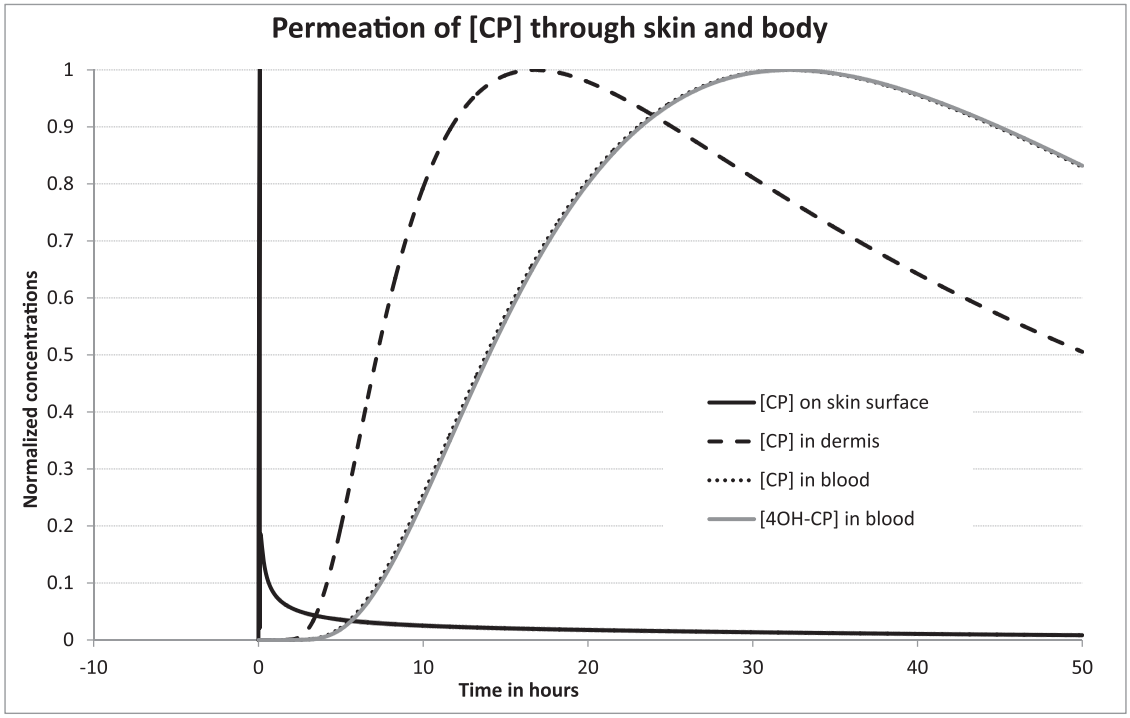
Dermal uptake

To explain the dermal absorption of CP through the skin into the blood, the normalized CP concentration is presented in Fig. 3. This normalized concentration graphically represents the proportion of the concentration in each of the skin compartments, i.e. the external skin surface, at the barrier between stratum corneum and dermis, in the blood stream, and the concentration of 4OH-CP in the blood. The dermal absorption of CP appeared to be very slow compared with the subsequent metabolism of CP to 4OH-CP in the liver, and Fig. 3 shows that CP diffuses into the blood around 3–4 h after dermal exposure.

PBPK model

The compartmental flow model for dermal exposure to CP is illustrated in Fig. 2, with CP being metabolized in the liver to form 4OH-CP, which is transported to the tissues. Physiological parameter values and partition coefficients for CP and 4OH-CP are presented in Table 2. The cumulative amounts of phosphoramide mustard and acrolein in the bone marrow were estimated to be 12.3 nmol for an average oncology nurse in a Dutch hospital after a 40-year occupational dermal exposure to CP during daily activities (Table 3). The concentrations of CP and 4OH-CP in the blood of an oncology nurse were on average 0.44 pmol/l (range: 0.076–1.26 pmol/l) and 0.0055 pmol/l (range: 0.00094–0.015 pmol/l), respectively. Blood concentrations of CP and 4OH-CP varied over time due to the intervals between dermal exposures, but did not show an accumulation of CP or 4OH-CP in the blood over time, because of the relatively low levels of dermal exposure and large time intervals between dermal exposures (Table 3). The average level of unchanged CP in urine was 0.42 pmol (range: 0.17–2.36 pmol).

The worst-case scenario with maximum task frequencies and exposure intensities for each task resulted in cumulative amounts of phosphoramide mustard and acrolein in the bone marrow of 1.83 μmol. The average concentrations of CP and 4-hydroxy-cyclophosphamide in the blood for this scenario appeared to be 65.5 pmol/l and 0.83 pmol/l, respectively (Table 3).



3 Normalized dermal absorption over time of one exposure pulse of CP from the external surface of the stratum corneum (black line) to the dermis (long-dashed line) and into the blood stream (short-dashed line).

Table 3. Leukemia excess risk calculations for Dutch oncology nurses using an epidemiological cohort study among cancer patients with CP-related secondary malignancies

Treated patients (Greene *et al.*, 1986)	
CP intravenous dose	46.35 g in 12 months (=14.78 mmol/month)
Cumulative phosphoramide mustard + acrolein in bone marrow	5.02 mmol
Cumulative leukemia risk after 10 years	111 000 per million
Non-treated patients (Greene *et al.*, 1986)	
CP intravenous dose	0
Cumulative phosphoramide mustard + acrolein in bone marrow	0
Cumulative leukemia risk after 10 years	1000 per million
Resulting exposure–response relationship	
Excess leukemia risk	2.2% over 10 years per mmol phosphoramide mustard in bone marrow
Dutch oncology nurses (average exposed)	
Total dermal exposure to CP after 40 years	113.1 μg (=0.43 μmol)
Average blood concentration of 4OH-CP	0.0055 pmol/l (range: 0.0009–0.015 pmol/l)
Phosphoramide mustard + acrolein in bone marrow after 40 years	12.3 nmol
Excess lifetime leukemia risk at age 80	1.04 per million
Dutch oncology nurse (worst-case exposure)	
Total dermal exposure to CP after 40 years	16 890 μg (=64.69 μmol)
Average blood concentration of 4OH-CP	0.83 pmol/l (range: 0.77–0.88 pmol/l)
Phosphoramide mustard + acrolein in bone marrow after 40 years	1.83 μmol
Excess lifetime leukemia risk at age 80	154 per million

Leukemia risk

The epidemiological study (Greene *et al.*, 1986) reported results from a cohort of 111 patients with a median total CP administered dose of 46.35 g over a 12-month period. This dose was associated with a cumulative risk for leukemia of 11.1% after a 10-year follow-up period (Table 3). This administered dose was inserted into the PBPK model as a monthly administration of (46.35/12 =) 3.86 g (14.78 mmol) CP into the blood compartment, which resulted in a cumulative delivered dose of phosphoramide mustard and acrolein in the bone marrow of 5.02 mmol, after the 12-month treatment period (Table 3). The cumulative risk for control patients who did not receive chemotherapy was 0.1% (Greene *et al.*, 1986). By using this baseline risk, a linear dose-response curve could be constructed, resulting in a 2.2% 10-year cumulative leukemia risk per mmol phosphoramide mustard in bone marrow. The excess lifetime leukemia risk at age 80 for exposure during a 40-year period was estimated to be 1.04 per million oncology nurses, using linear extrapolation from high to low dose (Table 3). The worst-case dermal

exposure scenario resulted in an excess lifetime leukemia risk of 154 per million oncology nurses (Table 3).

DISCUSSION

Based on the elaborate PBPK model (presented here) in combination with pharmacodynamic data from cancer patients, the excess lifetime risk of developing non-lymphocytic leukemia for nurses in Dutch hospitals, who have been occupationally exposed to CP via the skin of hands for 40 years (in total 113.1 μg), was estimated to be 1.04 per million oncology nurses. This risk estimate could potentially increase to a maximum of 154 per million oncology nurses for nurses with the maximum exposure frequency and maximum intensity for all tasks (worst-case scenario; in total 16 890 μg on the hands). The kinetics and side effects have been well investigated and described for patients treated with CP, which in combination with the available quantitative task-based dermal exposure measurement data makes this study unique in this type of occupational risk assessment studies. The presented PBPK model together with the dermal absorption model have shown to be useful to estimate the delivered dose in target organs after dermal exposure and consequently the risk of occupational dermal exposure to antineoplastic drugs.

This modeling approach could also be used to estimate the risk of reproductive health effects in relation to exposure to CP. However, because of the higher prevalence of these reproductive health effects, in comparison with the carcinogenic effects, it is possible to investigate these effects in an epidemiological study (and this has been done (Fransman *et al.*, 2007b; Selevan *et al.*, 1985; Stücker *et al.*, 1990; Stücker *et al.*, 1993; Valanis *et al.*, 1999)), which is preferable over a modeling approach like the one discussed in this study.

The modeling approach in this study uses point (average) estimates for each of the parameters (i.e. dermal exposure estimates, dermal absorption, and pbpk parameters) and does not take into account the distribution of variability and uncertainty around these point estimates. When more information becomes available on each of these parameters, future studies could also include Monte Carlo simulations to elucidate the variability and uncertainty of the parameters values to derive confidence intervals for the leukemia risk estimates.

Estimated dermal absorption was based on the MW and octanol/water partition coefficient of the compound and thickness of the skin. The MW and the octanol/water partition coefficient are chemical specific, and therefore, only the variation in skin thickness between individual and between body locations can influence the dermal absorption rate in our calculation. The thickness of the skin of hands was estimated to be 18 μm, which has previously been used to estimate dermal absorption of the hands (Cleek and Bunge, 1993). Since the diffusion of CP through the skin is quadratically related to the thickness of the skin, variation of this parameter between individuals could have a large influence on the diffusion rate of CP through the skin. In addition, the skin of nurses may be damaged due to the frequent washing of hands during and after their daily activities, which might change the penetration of CP through the skin. Furthermore, using gloves can create a warm and sweaty atmosphere between hands and gloves, which could have resulted in a quicker uptake of CP through the skin. However, both the thickness and potential damage of the skin and the atmosphere between gloves and hands would only influence the velocity with which CP crosses the stratum corneum but would not alter the total cumulative amount of CP that reaches the bone marrow and would therefore not have influenced the risk estimates over the 40-year period. Since CP is rapidly absorbed from the external skin surface into the outer layer of the stratum corneum, a large proportion of CP has been absorbed into the stratum corneum before hands are washed (69% of contamination has been absorbed 5 min after dermal exposure; 78% after 10 min; and 83% after 20 min). This means that diffusion of CP through the skin barrier will continue after the hands have been washed.

The PBPK model was based on several assumptions. Tissue/blood partition coefficients of CP and 4OH-CP were calculated from the octanol/water partition coefficient of CP based on the methodology described by Balaz and Lukacova (1999), and metabolic and renal clearance rates of both CP and metabolites were estimated based on published patient data. The large interpatient variability in these values confirms that individuals can be different in metabolic activity due to different enzyme levels and other factors such as age, diet, etc. and can therefore differ in susceptibility for the adverse side effects of CP. The fractions of administered CP excreted unchanged in urine have been reported to range between 2 and 36%

(Sladek, 1988), which is in our model directly related to the metabolic activation of CP (average: 76%, range: 59–93%). This means that the metabolic activation of CP between individuals can deviate maximally around 20% from our estimate. Because the cumulative dose of phosphoramide mustard in bone marrow is linearly related to this metabolic activity of CP and the associated leukemia risk is linearly extrapolated from this cumulative dose in bone marrow, the leukemia risk can maximally deviate 20% from our estimated leukemia risk based on reported differences in metabolic activity between individuals.

CP induces its own metabolism after repeated administration over a period of several consecutive days, which may result in an increase in formation of activated metabolites over time in patients treated with CP of approximately 55 to 65% (de Jonge *et al.*, 2005). Since the activation of 4OH-CP into phosphoramide mustard and acrolein is limited by the formation rate of 4OH-CP from CP and the occupational dermal exposure levels to CP among nurses are relatively low compared with the patient treatment dose, this autoinduction will most likely not affect the quantities of activated metabolites among oncology nurses. This means that the leukemia risk estimate for oncology nurses could have been slightly overestimated because autoinduction may have caused the cumulative doses of phosphoramide mustard and acrolein in the bone marrow of CP-treated patients to be 65% higher than the estimated 5.02 mmol after 12-month treatment. This would have resulted in an estimated excess lifetime leukemia risk among oncology nurses with average dermal exposure of 0.63 per million.

The cumulative risk estimate among ovarian cancer patients presented by Greene *et al.* (1986) was unadjusted for potential confounders. The risk estimate presented by Greene *et al.* was among patients who had already developed ovarian cancer, and these patients were older (48% older than 55 years) than oncology nurses (average age = 38 years). However, the impact of these factors on the cumulative leukemia risk of 11.1% is unknown. When linearly extrapolated to 'healthy' oncology nurses, this could have resulted in an overestimation of the leukemia risk for oncology nurses. The linear extrapolation was based on a linear additive risk model in which the incidence rate of leukemia for exposed patients was assumed to be the sum of a baseline rate (estimated in unexposed patients) and a linear function of exposure. Alternatively, a relative risk model could have been used instead, in which the baseline rate is multiplied by an exponential function of exposure. This would have resulted in considerably higher estimated excess lifetime risks of 1.93 and 287 per million for average exposed and worst-case scenarios, respectively. The data presented by Greene *et al.* do not allow an informed choice between these models, but an additive model is generally believed to be somewhat more plausible from a biological standpoint.

The risk of non-lymphocytic leukemia among Dutch oncology nurses exposed via the skin to a relatively low dose (113.1 μg on the skin of hands after 40 years) of CP during a 40-year working period (long term) was estimated using linear extrapolation based on the leukemia risk among patients who were treated with CP during 1 year (short term) at high therapeutic dose (46.35 g in 12 months). Among treated patients, most of the leukemic disorders appeared shortly after cessation of CP treatment (88% within 3 years) (Greene *et al.*, 1986). Although extrapolation of the risk from high to low dose was assumed to be linear, the difference in risk between a short-term high-dose (patient) and a long-term low-dose exposure (oncology nurse) cannot easily be estimated. Despite the fact that duration of treatment and the total CP amount administered in patient studies are usually correlated, Greene *et al.* found some indications that especially short higher doses pose a higher risk compared with lower prolonged exposures. Another issue is the assumption that exposures received at age 20 still affect the probability of contracting leukemia at age 70, while risk in the epidemiological study that was used to estimate the exposure disease relation quantitatively was calculated over a much shorter time period (10 years). Although this is not unusual in risk assessment, the effect would likely be that risks are overestimated.

The estimated lifetime risk of leukemias was previously estimated to be between 95 and 600 per million at age 70 (Sessink *et al.*, 1995), which is somewhat higher than the leukemia risk that we estimated. However, Sessink and co-workers estimated the daily uptake of CP based on excretion of unchanged CP in urine of healthcare workers and did not take into account the fact that CP is an inactive prodrug, which is metabolized in the liver to form active metabolites. Our study benefits from the availability of quantitative

dermal exposure measurement data, which enabled the estimation of dermal absorption, formation of active metabolites, and subsequent delivered dose of active metabolites in the target organ. In addition, the mean daily urinary excretion of 0.18 μg CP (range: 0.01–0.53 μg) described in the study by Sessink and co-workers is more than thousand fold higher than we estimated in our PBPK model (average: 0.11 ng CP, range: 0.04–0.62 ng), which was closer to the average level of urinary excretion that has been assessed more recently (Fransman *et al.*, 2007a), reflecting that exposure levels (and associated leukemia risks) among nurses in Dutch hospitals have decreased over time.

In the Netherlands, a maximum tolerable excess risk of one extra death by cancer per 250 deaths after 40 years of occupational exposure is used as a benchmark in risk assessment, and the target risk is set to one extra death by cancer per 25 000 deaths after 40 years of occupational exposure. The risk in this study was estimated to be 1.04 per million after 40 years of occupational exposure, which is well below the maximum tolerable risk and below the target risk. In the worst-case scenario (in which a nurse is assumed to perform each task with the maximum frequency without using protective gloves), the maximum annual risk in this study was estimated to be 154 per million after 40 years of occupational exposure, which is still below the maximum tolerable risk, but above the target risk. We need to emphasize that this risk estimate is solely for CP. CP is one drug out of a wide variety of antineoplastic drugs, of which a large proportion has been related to carcinogenic potential. To further elucidate the cancer risk for oncology nurses in Dutch hospitals working with all antineoplastic drugs, the effect of exposure of more (alkylating) antineoplastic drugs and their interactions should be investigated.

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