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Drugs Hazardous to Healthcare Workers

Evaluation of Methods for Monitoring Occupational Exposure to Cytostatic Drugs

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Abstract

We review the literature concerning possible health risks for individuals (e.g. healthcare workers and pharmaceutical plant employees) occupationally exposed to cytostatic drugs. Cytostatic drugs possess toxic properties and may therefore cause mutagenic, carcinogenic and teratogenic effects. Hence, individuals handling these drugs in the course of their employment may face health risks. For this reason, it is important to monitor occupational exposure to these drugs.

An overview of exposure monitoring methods is presented and their value is discussed. Most studies involve nonselective methods for biological monitoring and biological effect monitoring, such as the urinary mutagenicity assay and analysis of chromosomal aberrations and sister-chromatid exchanges in periph-

eral blood lymphocytes. The disadvantages of these biological methods are that their sensitivity is low and it cannot be proved beyond any doubt that the results found were caused by occupational exposure to cytostatic drugs. For occupational health services it is important to have sensitive and specific methods for monitoring exposure to cytostatic drugs. One of the most promising methods seems to be the determination of cyclophosphamide in urine using gas chromatography–tandem mass spectrometry.

Several studies have demonstrated exposure to cyclophosphamide and other cytostatic drugs, even when protective measures were taken and safety guidelines were followed. To estimate the magnitude of any health effects arising from this exposure, we calculated the risk of cancer due to occupational exposure to cyclophosphamide on the basis of available human and animal dose-response data and the amounts of cyclophosphamide found in urine. The initial results show an extra cancer risk for pharmacy technicians and nurses.

1. Cytostatic Drugs

Cytostatic drugs are widely used in the treatment of cancer and some non-neoplastic diseases.[1,2] Depending on their mechanism of anticancer action, these drugs are divided into several categories, such as alkylating agents, antibiotics, antimetabolites, free radical generators and mitotic inhibitors (table I).[1-3] Alkylating drugs act by alkylating the DNA of tumour cells. Antibiotics interfere with the transcription of DNA and antimetabolites block the synthesis of DNA and RNA. Free radical generators produce reactive free radicals in the vicinity of DNA. Mitotic inhibitors act on the mitotic mechanism necessary for karyokinesis.

In therapy, cytostatic drugs are often used as a combination of drugs having different mechanisms of action. Many cytostatic drugs interfere with the DNA, or with DNA synthesis, of tumour cells. Consequently, the proliferation of these cells is decreased. However, because of their generally nonselective mode of action, normal (nontumour) cells may also be damaged, resulting in toxic adverse effects. Workers handling these drugs, such as pharmacists, pharmacy technicians and nurses involved in their preparation and administration, and workers employed in the synthesis and production of these products, may face health risks.

1.1 Toxic Effects

The acute toxic effects of many cytostatic drugs, such as irritation of skin, eyes and mucous membranes, alopecia, nausea, vomiting and diarrhoea, have frequently been observed in patients treated with these drugs. More severe toxicity may occur in several organs and tissues, such as bone marrow (leucopenia, anaemia and thrombocytopenia), liver, bladder, kidney and lung.[1,2,4,5] However, during occupational activities such as preparation and administration of these drugs, acute toxic effects have not been observed except for accidents in the course of which workers have been exposed to large amounts of spilled drugs.

Apart from the acute effects, cytostatic drugs have delayed adverse effects such as mutagenicity, teratogenicity and carcinogenicity.[3] Mutagenic effects have been observed in mammalian systems *in vitro* (animal and human cells) and *in vivo* (animals) for many (alkylating) cytostatic drugs.[6-10]

Teratogenic effects have been found in experimental animal studies for several cytostatic drugs.[6-9] In some epidemiological studies, increases found in the number of spontaneous abortions and malformations in the offspring of nurses have been suggested to be caused by occupational exposure to cytostatic drugs.[11,12] In addition, an association between menstrual dysfunction and handling of cytostatic drugs has been found.[13]

However, in other studies no increased risks were found for miscarriages, malformations, low birthweights or preterm births among the offspring of nurses handling cytostatic drugs during pregnancy and for spontaneous abortions among women working in the pharmaceutical industry with exposure to cytostatic drugs.[14,15]

Table I. Carcinogenicity of cytostatic drugs in relation to their mechanism of action

Cytostatic drug	CAS registry no.	IARC evaluation[a]
Alkylating agents		
Busulfan	55-98-1	1
Carmustine	154-93-8	2A
Chlorambucil	305-03-3	1
Chlormethine	51-75-2	2A
Chlormethine *N*-oxide	126-85-2	2B
Chlornaphazine	494-03-1	1
Chlorozoticin	54749-90-5	2A
Cisplatin	15663-27-1	2A
Cyclophosphamide	50-18-0	1
Dacarbazine	4342-03-4	2B
Ifosfamide	3778-73-2	3
Lomustine	13010-47-4	2A
Melphalan	148-82-3	1
Semustine	13909-09-6	1
Streptozotocin	1883-664	2B
Thiotepa	52-24-4	1
Tresulphan	299-75-2	1
Antibiotics		
Azacitidine	320-67-2	2A
Bleomycin	11056-06-7	2B
Daunorubicin	20830-81-3	2B
Doxorubicin	23214-92-8	2A
Mitomycin	50-07-7	2B
Antimetabolites		
Fluorouracil	51-21-8	3
Mercaptopurine	50-44-2	3
Methotrexate	59-05-2	3
Free radical generator		
Azathioprine	446-86-6	1
Mitotic inhibitors		
Vinblastine	143-67-9	3
Vincristine	2068-78-2	3
Miscellaneous		
Procarbazine	366-70-1	2A

a 1 = carcinogenic to humans; 2A = probably carcinogenic to humans; 2B = possibly carcinogenic to humans; 3 = not classifiable as to its carcinogenicity to humans.[6-8]

CAS = Chemical Abstracts Service; **IARC** = International Agency for Research on Cancer.

According to the International Agency for Research on Cancer (IARC), there is sufficient evidence for the carcinogenicity in humans of 9 (alkylating) cytostatic drugs (group 1) [table I].[6-9] These conclusions are based on epidemiological studies showing secondary tumours in cancer patients treated with these drugs and primary tumours in noncancer patients treated with the drugs for other purposes.[16,17] In addition, several cytostatic drugs are carcinogenic in animal studies. They are categorised by the IARC as probably or possibly carcinogenic in humans (group 2A and group 2B, respectively) [table I]. A few cytostatic drugs (antimetabolites and mitotic inhibitors) are not considered by the IARC as classifiable as to their carcinogenicity in humans (group 3) [table I].

Based on current scientific knowledge of the mechanism of action of genotoxic carcinogens, among which are almost all alkylating cytostatic drugs, it is unlikely that there is any level of exposure that can be predicted to cause no adverse effects. Hence, exposure to these compounds has to be avoided.

1.2 Occupational Exposure

During the preparation and administration of cytostatic drugs there are several potential events, for example needlestick injuries and broken ampoules, that may result in exposure of hospital workers. Contaminated vials and ampoules are also a source of exposure. Contamination already present on vials and ampoules before preparation has started will result in further dissemination of the drug, especially when the cleaning procedures are ineffective and inefficient. Several studies have shown that the gloves used during preparation are permeable to cytostatic drugs, resulting in possible uptake of the drugs.[18-23] During preparation and administration of the drugs, overpressure will result in the release of aerosols. In addition, the urine, faeces, vomit, sweat, bedding and clothes of the patients may be contaminated with the drugs. This means not only a potential risk for nursing staff but

also for workers in the laundry and internal transport services and for family and friends of the patient. In addition, most nurses prefer not to use protective measures such as special clothes, masks and gloves in order to treat the patient in a friendly way.

About 15 years ago, it was shown that, compared with the use of a horizontal flow hood, the use of a vertical flow safety cabinet resulted in decreased mutagenic activity in the urine of technicians preparing cytostatic drugs.[24] After that, several publications suggested the possible risks to healthcare workers involved in the preparation and administration of cytostatic drugs. Consequently, protective measures were taken and safety guidelines were developed to protect the workers handling these drugs.[25-27] Special attention was given to the effective use of protective clothes, masks and gloves. In addition, preparation and administration procedures were improved and the vertical laminar airflow hood was introduced. However, in a recently published study, the effectiveness of the vertical laminar airflow hood was questioned when using cyclophosphamide.[28] A very low vapour pressure for this drug was observed at room temperature. It is supposed that molecules of vaporised cyclophosphamide are much smaller than the pore size of the high-efficiency particulate air (HEPA) filters and would not be retained in the vertical laminar airflow hood. Therefore, the reliance on vertical laminar airflow hoods to provide total protection from exposure to antineoplastic agent may be misleading and may provide a false sense of security. If this is true, then health care workers could be exposed to vapours of cyclophosphamide (and possibly other drugs) by the inhalation of contaminated air.

2. Monitoring of Occupational Exposure to Cytostatic Drugs

For workers handling cytostatic drugs, the ideal situation is complete prevention of exposure, because replacement of these agents by other less harmful drugs is not yet possible.[29,30] Second in priority is reduction in exposure by the use of closed systems. Recently, the results of a study were presented showing a dramatic reduction in environmental contamination using a closed system.[31,32] Finally, if all possibilities fail and are not appropriate, exposure should be reduced by using personal protective measures combined with the application of guidelines. In all cases the employer should offer workers all necessary information to achieve a safe working situation.

Despite the use of protective measures, it is still necessary to check whether there is exposure. In occupational health, several techniques are available to monitor exposure, dose or effect. Environmental monitoring and biological monitoring are used to measure environmental exposure and uptake (dose), respectively. Biological effects of drugs are assessed by biological effect monitoring or health surveillance.

2.1 Environmental Monitoring

Determination of air concentrations of cytostatic drugs (aerosols) is used for the quantification of external exposure to these drugs. Environmental air is sucked through a filter and the filters are extracted and analysed for the presence of cytostatic drugs. Using this procedure, cytostatic drugs were detected in the environment during the manufacturing process, during drug production, preparation and administration and during the treatment of laboratory animals.[18,33-44] In some studies, no cytostatic drugs were detected in the air.[36,37,45,46] However, it should be noted that the detection limit of the analytical method used will influence the results.

In several studies, wipe samples were taken and analysed from different surfaces (safety cabinets and floors in production, preparation and administration rooms) and objects (tables and vials).[18,39-44] Contamination was found in several areas. In addition, gloves and sleeve protectors for personal protection were frequently contaminated.

2.2 Biological Monitoring

Methods for biological monitoring can be divided into two groups: compound-selective methods and nonselective methods. For compound-

selective methods, the amount or the concentration of a particular compound or its metabolites is determined by using sensitive analytical chemical methods. For nonselective methods, common properties of a group of chemicals are measured, such as mutagenicity or electrophilicity. For biological monitoring of occupational exposure to cytostatic drugs, nonselective methods have been used very frequently.[47,48]

2.2.1 Urinary Mutagenicity Assay

Some cytostatic drugs are alkylating compounds and express their cytostatic activity by interaction with DNA, resulting in a covalent bond. This interaction may result in the occurrence of mutations, which can be detected in several bacterial and eukaryotic test systems. An assay frequently used to detect the mutagenic properties of chemical compounds is the so-called Ames assay. Application of the Ames assay to urine extracts of workers exposed to mutagenic compounds may indicate exposure to such compounds. However, no increase in mutagenicity does not mean that no uptake of these compounds occurs.[49,50] Therefore, this approach should be considered as a signal test.

In table II an overview of studies is presented in which urinary mutagenicity is used as an indicator of exposure to cytostatic drugs. In several studies, an increase in urinary mutagenicity was observed, whereas other studies showed no increase. In this nonselective method of biological monitoring, other factors in addition to exposure to cytostatic drugs, such as diet and exposure to environmental mutagens, may influence the results.[77-79] Together, these factors cause a variation in background levels of mutagenicity and it is questionable whether current levels of exposure to cytostatic drugs will result in a significant increase in urinary mutagenicity.[38,59] This might be a possible explanation for the negative results of some studies. In several studies an effect of smoking was observed.[54,61,63,65,70] The influence of smoking can be eliminated by the use of bacterial strains sensitive to cytostatic drugs but not to urinary mutagens caused by smoking. In 1 study, no correlation was found between mutagenicity and cyclophosphamide concentration in urine.[80]

In 1985, a large investigation was started in 3 Dutch hospitals to study the mutagenicity in urine of healthcare workers handling cytostatic drugs.[67]

Table II. Overview of studies in which urinary mutagenicity has been used as a marker of exposure to cytostatic drugs

Exposed group	Result	Reference
Nurses	+	Falck et al.[51]
Pharmacists	–	Staiano et al.[52]
Pharmacy technicians	–	Wilson & Solimando[53]
Pharmacy personnel	+	Anderson et al.[24]
(Smoking) nurses	+	Bos et al.[54]
Pharmacy technicians	+	Nguyen et al.[55]
Pharmacists	+	Theiss[56]
Nurses	–	Hoffman[57]
Nurses	–	Kolmodin-Hedman et al.[58]
Pharmacy technicians	+	Gibson et al.[59]
Nurses/pharmacists	–	Venitt et al.[60]
Nurses	–	Barale et al.[61]
Nurses	–	Cloak et al.[62]
Nurses/pharmacists/ pharmacy technicians	–	Everson et al.[63]
Nurses	+	Benhamou et al.[64]
(Smoking) nurses/pharmacy technicians	+	Breed et al.[65]
Pharmacy technicians	–	Connor et al.[66]
Nurses/pharmacy technicians	–	Fransman et al.[67]
Nurses	–	Friederich et al.[38]
Pilot plant workers, chemists, laboratory assistants	+	Pohlová et al.[68]
Nurses	+	Stucker et al.[69]
(Nonsmoking) nurses	+	Courtois et al.[70]
Nurses	+	Caudell et al.[71]
Nurses	–	Poyen et al.[72]
Nurses/oncology personnel	–	Poyen et al.[73]
Nurses/pharmacists	–	Sorsa et al.[36,37]
Process/production workers, laboratory technicians	–	Sorsa et al.[36,37]
Nurses	–	Krepinsky et al.[74]
Nurses	+	Thiringer et al.[75]
Mainly nurses and doctors	–	DeMéo et al.[76]

+ = Urinary mutagenicity was significantly higher in the exposed group than in the control group; **–** = there was no significant difference in urine mutagenicity between the exposed group andthe control group.

No increase in urinary mutagenicity was found in the exposed group when compared with the control group. However, in 1981 and 1985 an increase in urinary mutagenicity had been observed in nurses and pharmacy technicians working in Dutch hospitals.[54,65] The introduction of protective measures and safety guidelines are possibly the reasons why exposure to cytostatic drugs could no longer be detected in the later study.[67]

2.2.2 Thioether Assay

The determination of thioethers in urine is also a nonselective method for biological monitoring. The assay is used as an indicator for exposure to (potential) electrophilic compounds. Some cytostatic drugs have or develop electrophilic (alkylating) properties which may result in a reaction with glutathione. Mercapturic acids or thioethers can be formed and excreted in urine. Thus, an increase in thioether excretion is associated with exposure to (potential) alkylating compounds.

Five studies have been published in which the thioether assay was used to study occupational exposure to cytostatic drugs. In 3 studies an increase in thioethers was observed in the urine of nurses handling cytostatic drugs.[67,81,82] No safety measures were taken in 1 of these studies.[81] In 2 studies no increase in thioethers was observed in the urine of nurses,[83,84] although most of them did not wear gloves and masks.

In summary, the thioether assay is a rather nonsensitive method and the presence of a background is strongly influenced by smoking.[83] Hence, the thioether assay is not the ideal method for biological monitoring of occupational exposure to cytostatic drugs.

2.2.3 Analytical Chemical Methods

Determination of cytostatic drugs or their metabolites in blood or urine to measure occupational exposure belongs to the compound-selective methods of biological monitoring. Due to chemical reactivity, a rather complex biotransformation pattern and an expected low exposure level, it is reasonable to assume that only low concentrations of cytostatic drugs or their metabolites will be present in urine or blood. Hence, sensitive methods are necessary.

In several studies, cyclophosphamide and ifosfamide were detected in the urine of healthcare workers involved in the preparation and administration of these and other cytostatic drugs (table III).[18,19,39-41,80,85-90] After derivatisation with trifluoroacetic anhydride, cyclophosphamide and ifosfamide were determined with gas chromatography and nitrogen-phosphorus detection, electron-capture detection, mass-selective detection or (tandem) mass spectrometry. Detection with (tandem) mass spectrometry is favoured due to its high specificity and sensitivity (0.1 ng/ml of urine).[89,91] The results of the several studies show that workers were exposed to cyclophosphamide and ifosfamide, even if protective measures were taken and procedures were followed according to guidelines.

Although special guidelines and protective measures have been introduced to protect healthcare workers during the handling of cytostatic drugs, it was found that they do not prevent the uptake of cyclophosphamide and ifosfamide. Therefore, in one study additional protective measures were introduced.[19] The measures included adaptations of the laminar downflow hood, the use of special masks and wearing double pairs of gloves by the workers. The effects of these additional measures were compared with the results of a previous study.[18] It was shown that the introduced additional protective measures reduced the external exposure to cyclophosphamide. However, the mean urinary excretion rate of cyclophosphamide before and after the intervention was not statistically different.

In 4 studies, platinum was found in the urine or blood of nurses and pharmacists handling cisplatin. In 1 study, atomic absorption spectrophotometry was used as a detection method.[60] However, platinum was found in equal amounts in the urine of the control group of office workers. In the other studies, platinum concentrations were determined by voltametric analysis after ultraviolet photolysis.[46,90,92] Urinary platinum was significantly in-

Table III. Overview of studies of the urinary excretion of cyclophosphamide in (healthcare) workers exposed to cytostatic drugs

Exposed group	No. of workers	Period of urine sampling (days)	Mean (range) urinary excretion rate of cyclophosphamide (μg/day)[a]	Reference
Nurses	2	57[b]	0.47 (0.43-0.51)[c]	Hirst et al.[85]
Healthcare workers	20	4	0.39 (0-2.5)	Evelo et al.[80]
Pharmacy technicians	2	2	0	Sessink et al.[39]
Pharmacy technicians/nurses	18	1-2	0.05 (0-0.5)	Sessink et al.[40]
Animal keepers	4	2-5	0.05 (0-0.2)	Sessink et al.[41]
Pharmacy technicians[d]	9	1-2	1.36 (0-10.05)	Sessink et al.[18]
Pharmacy personnel/nurses	21	1-5	5.2 (0-38)	Ensslin et al.[86]
Nurses	8	1	0.79 (0-2.9)	Sessink et al.[87]
Pharmacy technicians	1			
Cleaners	2			
Pharmacy technicians	8	8-16	0.18 (0.01-0.53)	Sessink et al.[88]
Nurses	7	2-4	0.80 (0-4.2)	Sessink[89]
Pharmacy technicians[d]	9	5	0.16 (0-0.51)	Sessink et al.[19]
Pharmacists/pharmacy technicians	13	1	1.08 (0-9)	Ensslin et al.[90]

a Rates below the detection limits were set at zero.

b In both nurses urine sampling was performed over a total of 57 days.

c Mean and range were calculated by assuming a mean sampling period of 28.5 days.

d The same persons.

creased in a few urine samples of healthcare workers compared with a nonexposed control group.

In a study of urine and blood samples from nurses involved in the preparation of cyclophosphamide, methotrexate, fluorouracil, doxorubicin and cisplatin,[38] none of the urine samples contained measurable amounts of cisplatin. No methotrexate was detected in the blood samples.

Methotrexate has also been determined in the urine of nurses preparing methotrexate infusions and involved in the care of patients.[93] Concentrations were determined by high performance liquid chromatography and ultraviolet detection. The highest cumulative urinary excretion was observed in nurses preparing the methotrexate infusion, but traces of methotrexate were also detected in the urine of nurses engaged exclusively in the care of patients.

Exposure to methotrexate and fluorouracil was monitored in 2 pharmaceutical plants where workers are involved in the production of these drugs.[42-44,89] For the determination of methotrexate in urine, a fluorescence polarisation immunoassay method was developed.[42] Fluorescence polarisation immunoassay is frequently used for monitoring serum drug concentrations in patients treated with methotrexate. The assay was modified in such a way that methotrexate could be measured quickly and efficiently after solid phase extraction of urine samples from exposed workers. The excretion rate of methotrexate was higher for the exposed groups of workers compared with control individuals.

A gas chromatography–tandem mass spectrometry method was developed for the determination of α-fluoro-β-alanine, the main metabolite of fluorouracil in urine.[43,89] Before analysis, α-fluoro-β-alanine is derivatised with *S*-ethyltrifluorothioacetate in a sodium hydroxide solution followed by derivatisation in acidified n-butyl alcohol. α-Fluoro-β-alanine was detected in urine samples from several workers.[43,44]

In summary, several methods have been developed and validated for biological monitoring of occupational exposure to cyclophosphamide, ifosfamide, fluorouracil, cisplatin and methotrexate.

The best validated and most specific and sensitive method seems to be the determination of cyclophosphamide in urine. Since there is no background with this method, a non-exposed control group is not necessary. The results of several studies have shown that during the preparation and administration of cytostatic drugs, including cyclophosphamide, the workers were internally exposed to this particular drug. Uptake of cyclophosphamide was even found in pharmacy technicians involved in the preparation of cytostatic drugs other than cyclophosphamide.[18,40] The exact exposure routes could not be identified, although it was concluded that inhalation was probably not the only route involved. These results, especially those showing urinary excretion of cyclophosphamide by healthcare workers not involved in the preparation or administration of this drug, demonstrate the value of this biological monitoring method.

2.3 Biological Effect Monitoring

During the last 10 years, rapid development of new methods to detect the early, possibly reversible, biological effects of mutagenic and carcinogenic compounds has taken place. These methods are generally termed biological effect monitoring. Among these assays are cytogenetic methods such as analysis of chromosomal aberrations, sister-chromatid exchanges and micronuclei proliferation in blood lymphocytes. More recently, methods have been developed to detect primary DNA damage.

Effects using these assays have been observed in *in vitro* and animal studies dealing with factors such as radiation and alkylating chemicals. Cytogenetic methods are now frequently used as markers of occupational exposure to cytostatic drugs.[47,48]

2.3.1 Cytogenetic Methods

Table IV shows an overview of studies performed with cytogenetic methods in healthcare workers handling cytostatic drugs. In most studies no increase in cytogenetic effects was observed. As with the mutagenicity assay, the cytogenetic methods are nonselective, meaning that the effects registered could also be caused by other factors. The effects measured by sister-chromatid exchanges and chromosomal aberrations are cumulative, and therefore the effect measured at a particular time is due to total exposure in the past. Age is a confounder when using micronuclei proliferation because micronuclei increase with age.[36] The causal relationship between cytogenetic effects and ad-

Table IV. Overview of studies in which cytogenetic methods and primary DNA damage in blood lymphocytes of healthcare workers have been used as markers of occupational exposure to cytostatic drugs

Parameter	Result	Reference
SCE	+	Norppa et al.[94]
CA and SCE	+	Waksvik et al.[95]
SCE	–	Szigeti et al.[96]
SCE	–	Kolmodin-Hedman et al.[58]
CA and SCE	–	Stiller et al.[97]
CA	+	Nikula et al.[98]
SCE	–	Barale et al.[61]
SCE	–	Jordan et al.[99]
CA and SCE	+	Pohlová et al.[68]a
CA and SCE	–	Stucker et al.[69]
CA and SCE	–	Benhamou et al.[100]
CA and SCE	–	Sorsa et al.[36]b
MN	?	Sorsa et al.[36]b
CA	+	Oestreicher et al.[101]
SCE	–	Oestreicher et al.[101]
CA and SCE	–	Sarto et al.[82]
CA and SCE	–	Krepinsky et al.[74]
CA and SCE	+	Milkovic-Kraus et al.[102]
CA	–	Cooke et al.[103]
SCE	+	Sardas et al.[104]
SCE	+	Thiringer et al.[75]
CA	+	Grummt et al.[105]
CA	+	Sessink et al.[87]
Primary DNA damage	+	Fuchs et al.[106]
Primary DNA damage	+	Oesch et al.[107]
SCE	+	Brumen et al.[108]
SCE and MN	–	Ensslin et al.[90]

a Pilot plant workers, chemists and laboratory assistants.

b Pharmacists, nurses, process and production workers and laboratory technicians.

CA = chromosomal aberrations; **SCE** = sister-chromatid exchanges; **MN** = micronuclei proliferation; **+** indicates that the parameter in question was significantly higher in the exposed group compared with the control group; **–** indicates that there was no significant difference in the parameter in question between the exposed group and the control group; **?** indicates that the results are inconclusive.

verse health effects is unknown. The usefulness of the cytogenetic methods is limited by a large variable background level. In addition, these methods are highly time consuming.

To study a possible relationship between drug uptake and an early biological response in healthcare workers, the excretion of cyclophosphamide in urine and the presence of chromosomal aberrations in peripheral blood lymphocytes was determined in 4 groups of participants with various levels of exposure status (Dutch and Czech healthcare workers handling cytostatic drugs and Dutch and Czech control healthcare workers not handling these drugs).[87] The groups were subdivided into smokers and nonsmokers. The results suggested an additive effect of exposure and smoking in the Dutch participants and a more than additive effect in the Czech participants. In the urine samples of the Dutch workers exposed to cyclophosphamide, the cyclophosphamide excretion rate ranged from 0.1 to 0.5 μg/24 hours. Higher excretion rates were found for the exposed Czech workers, ranging from 0.1 to 2.9 μg/24 hours. On an individual basis, no correlation was observed between the amounts of cyclophosphamide excreted in urine and chromosomal aberrations. The results suggest that urinary levels of cyclophosphamide of up to 2.9 μg/24 hours may result in long-term biological effects such as chromosomal aberrations. Since chromosomal aberrations are considered to be indicators of an increased genetic risk, these urinary cyclophosphamide concentrations may not only demonstrate absorption of cyclophosphamide but may also indicate a serious risk, especially for smokers.

2.3.2 Point Mutations

Another technique for biological effect monitoring is the assessment of mutation frequencies [at the HGPRT (hypoxanthine-guanosine phosphoribosyltransferase) locus] in lymphocytes as an indicator of exposure to genotoxic compounds. In a study using this technique, the number of point mutations was increased in a group of nurses and pharmacists handling cytostatic drugs when compared with controls, in spite of the use of gloves, masks and a vertical flow safety cabinet.[109] Mutation frequencies were also increased in a group of industrial workers employed in cyclophosphamide manufacturing and drug formulation compared with a nonexposed population.[110]

2.3.3 DNA Damage

A higher rate of DNA strand breaks was found in nurses handling cytostatic drugs without adequate safety precautions compared with nurses handling cytostatic drugs with safety precautions and with a non-exposed control population.[106,107]

2.3.4 Renal Dysfunction

The early effect parameters retinol-binding protein and albumin in urine, as indicators of nephrotoxic effects, were determined in healthcare workers involved in the preparation and administration of cytostatic drugs.[111] No difference was found between the exposed group and the nonexposed control group. Although it was demonstrated that the healthcare workers were exposed to cyclophosphamide, as measured by the urinary cyclophosphamide excretion rate, the results show that these exposure levels did not cause any defined nephrotoxic effects.

2.3.5 Immunological Effects

Many cytostatic drugs are immunosuppressive drugs. In 1 study immune functions were used as markers of exposure for nurses handling cytostatic drugs.[112] No differences were observed between nurses and control participants with respect to immune functions. The investigators concluded that the parameters measured were not sufficiently sensitive.

3. Cancer Risk

3.1 Assessment

We have performed a cancer risk assessment of occupational exposure to cyclophosphamide, a genotoxic carcinogenic cytostatic drug, on the basis of data from an animal study and from reports of the frequency of primary and secondary tumours in patients treated with cyclophosphamide.[88] We realise that this is a first approach having several

pitfalls. The urinary excretion of cyclophosphamide in healthcare workers (mean of 0.18 μg/day) was used to estimate the uptake of cyclophosphamide, which ranged from 3.6 to 18 μg/day.

Based on the animal data, cancer risks were calculated for a healthcare worker with a bodyweight of 70kg over 40 years of work at 200 days/year (linear extrapolation). The lifetime risks (70 years) of urinary bladder cancer in men, and leukaemias in men and women, were found to be nearly the same and ranged from 95 to 600 extra cases per million workers.

Based on the patient studies, cancer risks were calculated by multiplication of the 10-year cumulative incidence per gram of cyclophosphamide in patients by the estimated mean total uptake in healthcare workers over 10 years at 200 days/year. Using linear extrapolation from the secondary tumour data, the risk estimates of leukaemias in women ranged from 17 to 100 extra cases per million workers over 10 years. Comparable results were estimated for the risk of urinary bladder tumours and leukaemias in men and women when primary tumour data were used. Thus, on an annual basis, the cancer risks obtained from both the animal data and the patient study were nearly the same and ranged from about 1.4 to 10 extra cases per million workers.

3.2 Legislation

In the US, Sweden, Germany and the European Union, threshold limit values or otherwise defined exposure levels have been introduced for some genotoxic carcinogens in order to protect workers.[113] In The Netherlands, it is proposed that a cancer risk for each compound of 1 extra case per million workers per year ('target risk') should be sought and that no risk higher than 100 cases per million workers per year ('prohibitory risk') should be accepted.[113]

From our estimates in section 3.1, it appears that in a number of Dutch studies the 'target risk' was exceeded but that the 'prohibitory risk' was not approached. Nevertheless, it should be noted that in some studies particular groups of workers have excreted higher amounts of cyclophosphamide than the mean daily excretion of 0.18μg used for our cancer risk assessment, possibly resulting in higher risks (table III). For example, in 1 study with 7 nurses a mean excretion rate of 0.8 μg/day was found.[89] This cyclophosphamide excretion rate, approximately 5 times higher than the mean, may result in a correspondingly higher cancer risk.

Although our assessment of cancer risk caused by exposure to cyclophosphamide has its limitations, the values calculated for cyclophosphamide exposure of healthcare workers indicate that, in spite of the protective measures that they use, these workers may have higher risks for cancer because of their handling of cyclophosphamide and/or other cytostatic drugs.

4. Conclusions

To assess occupational exposure to antineoplastic agents, several methods have been developed and applied. Most studies concern nonselective methods for biological monitoring and biological effect monitoring, such as the urinary mutagenicity assay and the analysis of cytogenetic endpoints. These methods are directed to the possible genotoxic risk. The more recently developed analytical chemical methods are developed and validated to establish occupational exposure. The sensitive method for the detection of exposure to cyclophosphamide has shown its suitability and applicability in the detection of low exposure levels. The value of this new sensitive biomonitoring method has been demonstrated by the detection of cyclophosphamide in the urine of healthcare workers who are not directly involved in the preparation and administration of cyclophosphamide.

Several studies have suggested a role for skin uptake in the internal exposure to cytostatic drugs. More recently, a possible role in uptake is suggested by the presence of the drugs in the vapour phase resulting in inhalation of the vapour. The methods mentioned above and especially the sensitive analytical chemical method for the analysis of cyclophosphamide in environmental samples and urine may be helpful in measuring occupa-

tional exposure, to find causes of exposure and, finally, to reduce exposure, because it is striking that despite the introduction of safety guidelines and protective measures, healthcare workers are still exposed to these toxic drugs.

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